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HIROKAZU OKAMOTO

**Development of Polyprenylazacycloalkanone Derivatives as
Percutaneous Penetration Enhancers and Their Mechanism
of Action Based on Diffusion Model**

A Thesis
Submitted to the Faculty
of
Pharmaceutical Sciences
of
Kyoto University

by HIROKAZU OKAMOTO

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General Introduction

The development of novel drug delivery systems has become a matter of great interest in the pharmaceutical field because they enable us to optimize drug utilization, to reduce local or systemic side effects, to improve patient compliance by the extended duration, and so on.¹⁾ Application of drugs to the skin has long history but has recently received increased attention because of the following advantages over other conventional therapeutic modality.²⁾

- 1) Gastrointestinal absorption problems in oral administration are avoided.
- 2) First-pass effect is avoided.
- 3) Pain caused by injection is avoided.
- 4) Long duration of action increases patient compliance.
- 5) Drugs with narrow therapeutic range or with short half-life can be delivered.
- 6) Better control of blood levels for long duration is possible.
- 7) Therapy can be quickly terminated by removing the device.

The transdermal therapeutic devices have already been exemplified by the systems of scopolamine, nitroglycerin, clonidine, estradiol, and isosorbide dinitrate.²⁾ Except for these drugs, however, few drugs penetrate through the skin rapidly enough to reveal their therapeutic effect because the outermost layer of skin, the stratum corneum, forms a strong barrier to most external substances including drugs. One approach to deliver an effective dose of drug through skin is to reduce temporarily the barrier properties of skin by the aid of penetration enhancers or accelerants.^{3,4)} Many compounds such as 2-pyrrolidone, dimethylsulfonamide, dimethylsulfoxide, decylmethylsulfoxide, and urea, and surfactants or fatty acids have been

reported to have activities for enhancing drug penetration through skin. Recently, considerable attention has been given to 1-dodecylazacycloheptan-2-one (Azone) as a potent penetration enhancer for a wide range of drugs.^{5,6)} However, the action mechanism of these compounds is unclear and the development of penetration enhancer with high activity and low skin irritancy is still in its infancy.

The percutaneous penetration rate of the drug is determined by the three factors such as the physicochemical properties of drug, those of vehicle, and nature of skin.⁷⁾ A pharmaceutical additive can affect both the nature of skin and the thermodynamic condition of a penetrant in vehicle, which often confuses the discussion on the effect of the enhancer.⁸⁾ So it is necessary to evaluate the effects of enhancers on the drug penetration through skin by separating an effect on the skin from that on the thermodynamic activity of drug in vehicle. Also the effect of enhancer should be varied by the properties of penetrant and vehicle and original condition of the skin.

The present study was carried out to clarify the following points through the examination of effects of polyprenylazacycloalkanone derivatives, which have the azacycloalkanone moiety as a mild polar group and an alkyl or alkenyl (terpene) chain as a hydrophobic tail chain, on the skin penetration of drugs.

- 1) evaluation of activity of polyprenylazacycloalkanone derivatives as percutaneous penetration enhancers
- 2) the relationship between the structure and activity of the penetration enhancer
- 3) the relationship between the physicochemical properties of penetrant and efficacy of enhancer
- 4) the relationship between the applied dose of enhancer and its efficacy
- 5) the relationship between the physicochemical properties of vehicle and

efficacy of enhancer

- 6) development of diffusion model which works effectively for analyzing and predicting percutaneous penetration process

The obtained penetration profiles were analyzed based on a diffusion model for the finite dose system. The effect of the enhancers will be discussed in physicochemical terms such as diffusion constant and partition coefficient.

Chapter 1. Development and Evaluation of Novel Percutaneous Penetration Enhancers

The transdermal route has many advantages for the administration of drugs in local and systemic therapy. However, the outermost layer of skin, the stratum corneum, forms a strong barrier to most external substances including drugs. One approach to deliver an effective dose of drug through skin is to temporarily reduce the barrier properties of the skin by the aid of penetration enhancers or accelerants.^{3,4)} Many compounds, such as 2-pyrrolidone, dimethylsulfonamide, dimethylsulfoxide, decylmethylsulfoxide, and urea, and surfactants or fatty acids have been reported to enhance drug penetration through the skin. Recently, considerable attention has been given to 1-dodecylazacycloheptan-2-one (Azone) as a potent penetration enhancer for a wide range of drugs, although its mechanism of action is unclear.^{5,6)} Azone has a structure which is considered to be a chemical combination of pyrrolidone and decylmethylsulfoxide. Both the long alkyl chain moiety and the mild polar head group of Azone seem to be necessary for its action as a penetration enhancer. Examination of the effects of these moieties on the action of penetration enhancer, therefore, should give useful information on the development of penetration enhancers.

In this investigation, terpenes, which are known to be endogeneous components of the skin, were chosen as the alkyl chain moieties and nine types of new percutaneous penetration enhancers with different azacyclo ring moieties were designed. This chapter will demonstrate the effect of these substances on the percutaneous penetration of mitomycin C (MMC) through the skin of hairless mouse and rat as the first step of the investigation.^{9,10)}

The favorable penetration enhancer should have low toxicity or irritancy

to the skin as well as high penetration enhancing activity. From this point of view, the primary skin irritation induced by the enhancers in rabbit was also examined.¹¹⁾

1.1. Experimental

1.1.1. Materials

MMC was obtained from Kyowa Hakko Kogyo Co., Japan. Azone was kindly supplied by Nelson Research Center, USA. The newly developed percutaneous penetration enhancers (Fig. 1), 1-geranylazacycloheptan-2-one (7GU), 1-farnesylazacycloheptan-2-one (7FU), 1-geranylgeranylazacycloheptan-2-one (7GGU), 1-(3,7-dimethyloctyl)azacycloheptan-2-one (7GS), 1-(3,7,11-trimethyldodecyl)azacycloheptan-2-one (7FS), 1-geranylazacyclohexan-2-one (6GU), 1-geranylgeranylazacyclohexan-2-one (6GGU), 1-geranylazacyclopentan-2,5-dione (5GUDO), and 1-farnesylazacyclopentan-2-one (5FU) were synthesized by Kuraray Co., Japan. All other reagents used were of analytical grade.

1.1.2. Determination of Lipophilic Index of Enhancer

The lipophilic index was determined by high-performance liquid chromatography (HPLC).¹²⁾ An HPLC system (TRI ROTAR, Japan Spectroscopic Co., Ltd.) equipped with a UV detector (UVIDEC-100-III, Japan Spectroscopic Co., Ltd.) operating at 210 nm was used in a reverse-phase mode. The stationary phase was silica gel bonded chemically with octadecyl chains prepacked into a 10 cm stainless steel column (Cosmosil 5C18 packed column, Nacalai Tesque, Inc., Japan). A mixture of methanol (95-75 %) and distilled water (5-25 %) was employed as the mobile phase. The elution time of a solvent (t_0) and the retention time of an enhancer (t_R) were determined at

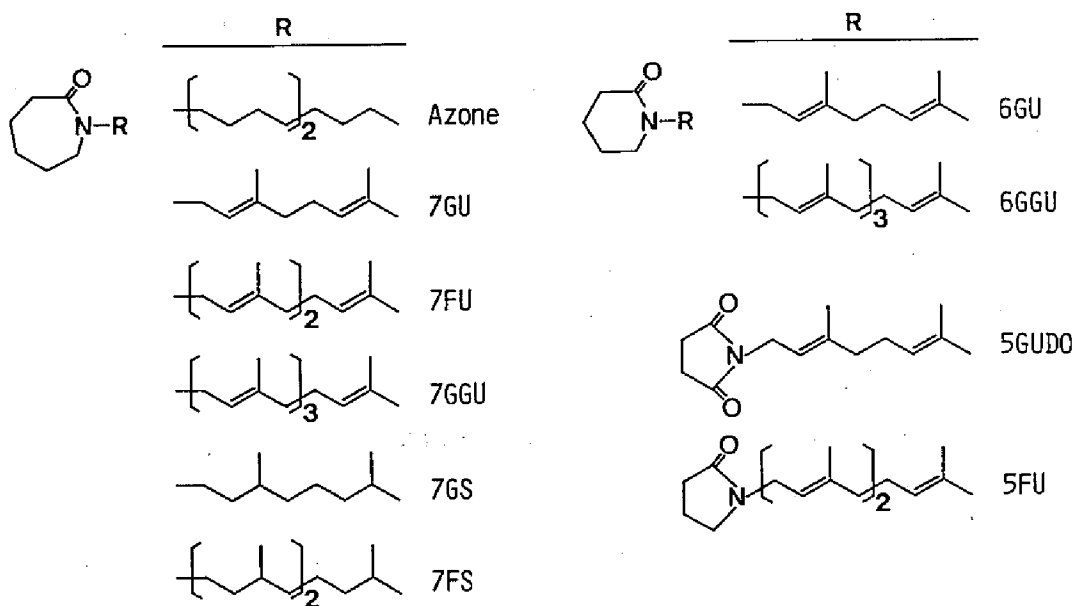


Figure 1. Structures of Azone and polyprenylazacycloalkanone derivatives.

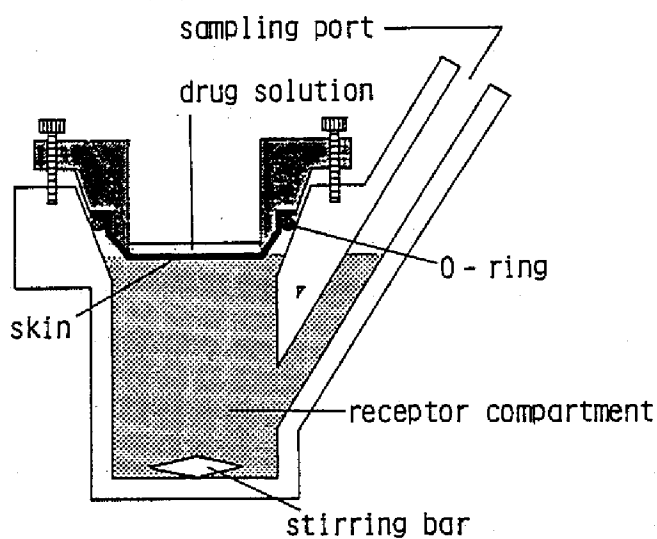


Figure 2. Sink type diffusion cell.

different mobile phase composition. The $\log k'$ value defined by Eq. 1 was plotted against the methanol concentration in the mobile phase and the extrapolated $\log k'$ value to 0 % methanol was obtained as an index of lipophilicity of enhancer ($\log k'_0$).

$$\log k' = \log[(t_R - t_0)/t_0] \quad (1)$$

1.1.3. In Vitro Percutaneous Penetration Experiment

Transdermal delivery rates of MMC were determined using an in vitro diffusion cell procedure. The full-thickness dorsal skin of male hairless mice (7-9 weeks) was removed in one piece and adherent fat and other visceral debris were removed carefully from the undersurface. Also, the full-thickness dorsal skin of male Wistar rats (230-250 g) was obtained after the removal of the hair with electric clippers, followed by the careful removal of adipose tissue. The freshly excised skin was mounted on a diffusion cell with an available diffusion area of 8.04 cm^2 (Fig. 2). The receptor compartment of apparatus was filled with 48 ml of saline containing 100 ppm of kanamycin sulfate. Test formulations were prepared by suspending MMC in ethanol or in 3.3 % ethanolic solution of an enhancer to a total concentration of 10 mM. One milliliter of MMC suspension was applied to each donor compartment (donor volume, ca. 19 ml). In all experiments, the donor cell was sealed with a silicone stopper to prevent evaporation of the test sample. The diffusion cell was placed in a thermostated chamber maintained at 37°C and the receptor medium was stirred with a magnetic stirrer. At appropriate intervals, 1 ml of the receptor medium was withdrawn and this volume was replaced with fresh medium. Diffusion experiments were carried out for 16 h with hairless mouse skin and for 30 h with rat skin. At the end of each experiment, the drug in the donor phase was recovered with ethanol (25 ml).

In the pretreatment experiments, 1 ml of ethanol or 3.3 % ethanolic solutions of enhancers were applied to the donor side of the rat skin for 24 h. After the removal of the solution and the washing of the surface of the skin three times with each 5 ml of ethanol, 2 ml of aliquots of a 2 mM MMC solution in ethanol were applied and the diffusion experiments were carried out as described above.

The concentration of MMC appearing in the receptor medium was measured by HPLC as described by Sasaki et al.¹³⁾ MMC recovered from the donor phase with ethanol was measured by spectrophotometric analysis at 360 nm after centrifugation and adequate dilution.

1.1.4. Acute Skin Primary Irritation Test

The dorsal hair of five albino rabbits (2.0–2.6 kg) was removed with electric clippers 24 h before the application of the test compounds. Each of the test compounds was saturated onto a paper disc and mounted on the aluminium disc of the Finn Chamber (Epitest, Helsinki, Finland). Test compounds were applied without any dilution to exclude the vehicle effect. A controller applied ten test compounds to the back of a rabbit with two pieces of Finn Chamber sheet, fixing five chambers in a row at a distance of 20 mm. A cotton bandage around the trunk of the rabbit secured the contact of the test compounds and the skin. After 24 h, the test samples were removed, and sites were evaluated for erythema and edema every 24 h for seven days using the Draize scoring system.^{14,15)} The grader had no information about the application of the test compounds. Primary irritation indices (PII) were calculated for erythema and edema by averaging the five daily scores.

1.2. Results

1.2.1. Structures and Properties of Enhancers

The structures of enhancers examined in this study are shown in Fig. 1. Hydrogenation of the double bonds in the side chain of 7FU yields the structure of 7FS, which can be considered as an Azone derivative methylated at three positions in its alkyl chain. Similarly, hydrogenation of the side chain of 7GU gives 7GS. The double bonds in 2 position in the geranyl, farnesyl, and geranylgeranyl chains are mixture of trans and cis form (trans : cis = 7 : 3). The double bonds in 6 position in the farnesyl and geranylgeranyl chains and that in 10 position in the geranylgeranyl chain are all trans form. All compounds are oil at room temperature and soluble in ethanol to make 3.3 % solutions but they are immiscible with water.

The plots of $\log k'$ values of enhancers against methanol concentration of the mobile phase show a reasonable linear relationship (Fig. 3). The lipophilic indices which were determined by the extrapolation of these plots to 0 % methanol are listed in Table I. The large value of lipophilic index means the high lipophilicity of the compound. Table I also lists the boiling points and the estimated solubility parameters which are calculated using the method of Fedors.¹⁶⁾ The solubility parameters express the cohesion between like molecules,¹⁷⁾ and is a good index of polarity of compounds, which is supported by its correlation with dielectric constants.¹⁸⁾ The solubility parameters inversely follow the order of the determined lipophilic indices.

1.2.2. Effect of Coadministration of Enhancers on Percutaneous Penetration of MMC

Figure 4 shows the effect of Azone, 7GU, 7FU, 5GUDO, and 5FU on the

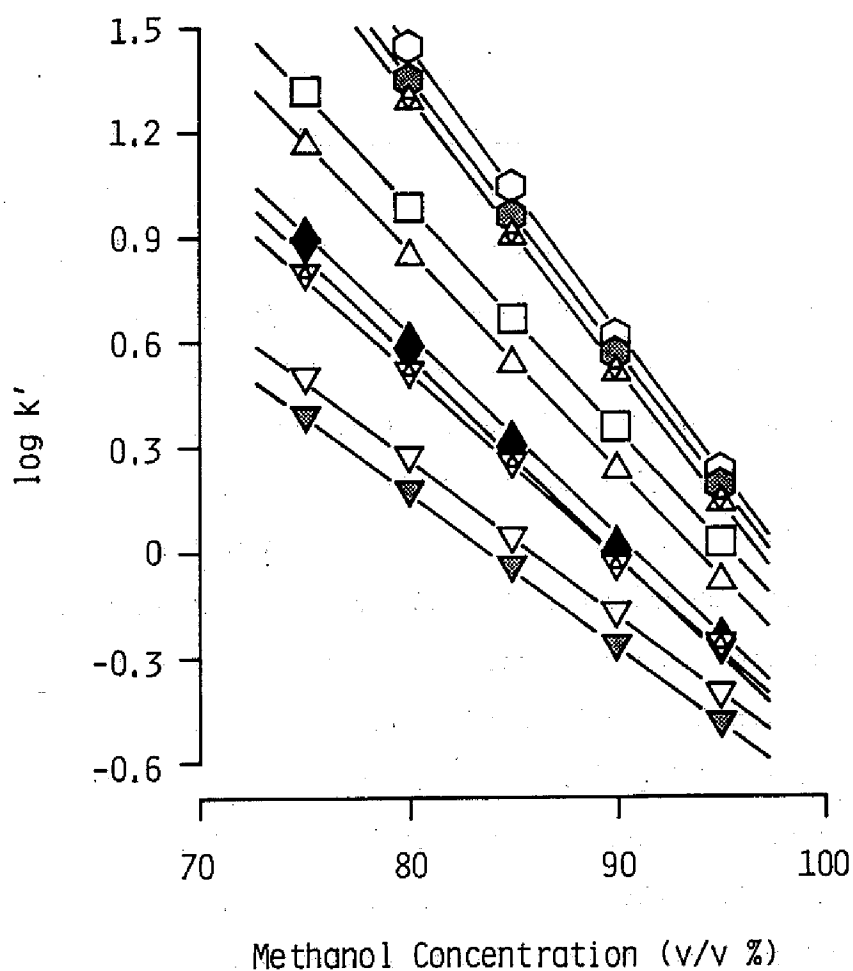


Figure 3. Relationship between $\log k'$ values of percutaneous penetration enhancers and methanol concentration (v/v %) in the mobile phase.

Key: (□) Azona, (▽) 7GU, (△) 7FU, (⬡) 7GGU, (▾) 7GS, (▴) 7FS, (▾) 6GU, (⬢) 6GGU, (▿) 5GUDO, and (▲) 5FU.

Table I. Physicochemical Properties of Polyprenylazacycloalkanone Derivatives

Compound	Molecular Weight	Solubility Parameter ^a (cal/cm ³) ^{1/2}	Lipophilic Index	Boiling Point (°C)
Azone	281	9.07	6.14	158-160 (0.1 mmHg) ^b
7GU	249	9.21	3.86	170 (0.3 mmHg)
7FU	317	9.10	5.86	210 (0.3 mmHg)
7GGU	385	9.02	7.94	ND ^c
7GS	253	8.95	4.78	140 (0.5 mmHg)
7FS	323	8.80	7.48	230 (0.3 mmHg)
6GU	235	9.25	3.69	138 (0.4 mmHg)
6GGU	371	9.04	7.57	225 (0.3 mmHg)
5GUDO	235	10.08	5.16	195 (0.3 mmHg)
5FU	289	9.15	5.23	205 (0.3 mmHg)

^a Estimated value by the method of Fedors.¹⁶⁾

^b Reported by Stoughton.⁵⁾

^c Not determined.

percutaneous penetration of MMC through hairless mouse skin in vitro. The penetration of MMC without enhancer was rather low and only 1.23 % and 3.53 % of the total amount was recovered in the receptor compartment at 12 h and 16 h, respectively. On the other hand, the penetration of MMC was greatly improved by the coexistence of Azone, 7GU, 7FU, and 5FU, and the amounts of MMC in the receptor phase were about 40 times at 12 h and 20 times at 16 h higher than the control in all cases. At 10 h, the highest penetration of 35.32 % of the applied dose in the receptor phase was produced by 7FU, and 5FU (31.54 %), Azone (19.51 %), and 7GU (16.79 %) followed this. 5GUDO also enhanced the penetration of MMC but at the time period of 12 to 16 h its effect was about a half of those of the other enhancers. The amount of MMC remaining in the donor phase at the end of experiment was 84.9 % for control, while enhancers reduced this amount to 2-5 % except for 5GUDO (Table II).

Figures 5A-C show the permeation profiles of MMC through rat skin when applied with or without enhancers. All newly developed enhancers as well as Azone markedly enhanced MMC penetration from ethanol through rat skin ($p < 0.001$ in all comparisons at 30 h). The effects of Azone, 7GU, 7FU, and 5FU were remarkable. When Azone was dissolved in isopropyl myristate vehicle, no enhancement was observed (data not shown).

The enhancers with a geranyl chain (7GU, 6GU, and 5GUDO) showed a longer lag time than the others. After the prolonged lag time, 7GU and 6GU produced almost the same penetration rate as the other enhancers, although the amount of penetrated MMC was equivalent to that of the other enhancers at 30 h. 5GUDO showed less effect.

Table II lists the amount of MMC remaining in the donor phase at the end of the experiment, which was significantly decreased by these compounds ($p < 0.001$ in all comparisons). In all diffusion experiments, the amount of

MMC disappearing from the donor phase fairly correlated with that appearing in the receptor phase.

The difference between the initial dose of MMC and the recovered amount (sum of those in the donor and receptor phase) of MMC should correspond to the amount of MMC existing in the skin at the end of the experiment or that of MMC degraded during the diffusion experiment. The decrease in the amount of MMC in the receptor phase observed in the Azone system may indicate decomposition of MMC. At the end of this experiment, a peak with shorter retention time than that of MMC was observed on a HPLC chart of the receptor medium, while this peak was much smaller than that of MMC. On the absorption spectra (240–420 nm) of the donor and receptor solutions, a clear peak of MMC was recognized at 360 nm and a small peak was observed at 280 nm. Thus, the decomposition of MMC seems to be minor and does not lead to misinterpretation of the enhancing activity.

1.2.3. Percutaneous Penetration of MMC through Rat Skin Pretreated with Enhancers

In the previous series of experiments, the initial thermodynamic activity of MMC in the donor phase was maintained constant by employing suspension formulations. Therefore, the increase in MMC penetration is considered to be resulted from direct effects of the enhancers on the skin. In order to further elucidate this problem, the effects of the enhancers such as 7GU, 7FU, 5FU, 5GUDO, and Azone, were examined by pretreatment experiments and the results are shown in Fig. 6. Pretreatment of rat skin with Azone resulted in a rapid increase in MMC penetration after the application of MMC solution. On the other hand, appearance of MMC in the receptor phase was delayed after pretreatment with 7GU, 7FU, and 5FU compared with the pretreatment with Azone.

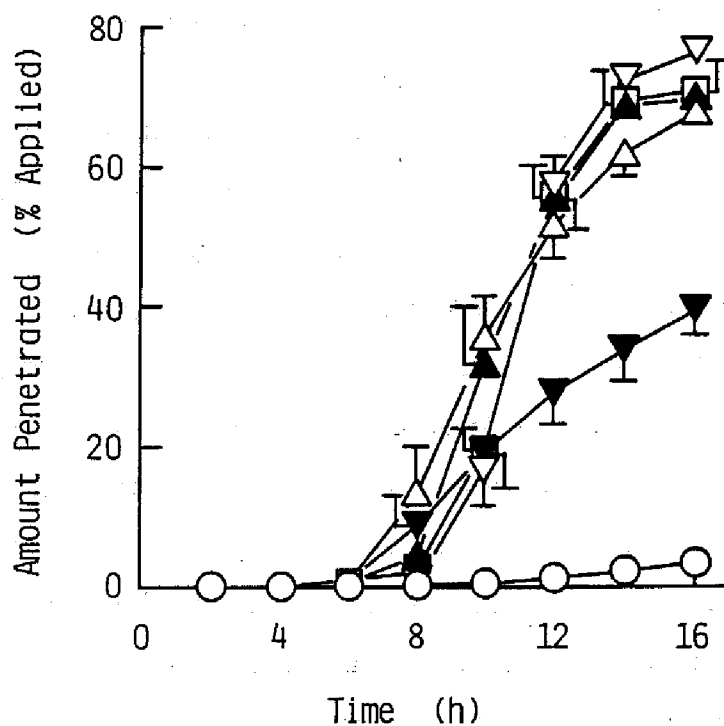


Figure 4. Penetration of MMC through hairless mouse skin at 37 °C. One milliliter of MMC suspensions (total concentration, 10 mM) in ethanol (○) or in 3.3 % ethanolic solutions of Azone (□), 7GU (▽), 7FU (△), 5GUDO (▼), or 5FU (▲) were applied. Each point represents the mean value of 3-4 experiments and vertical bars indicate standard errors of the mean.

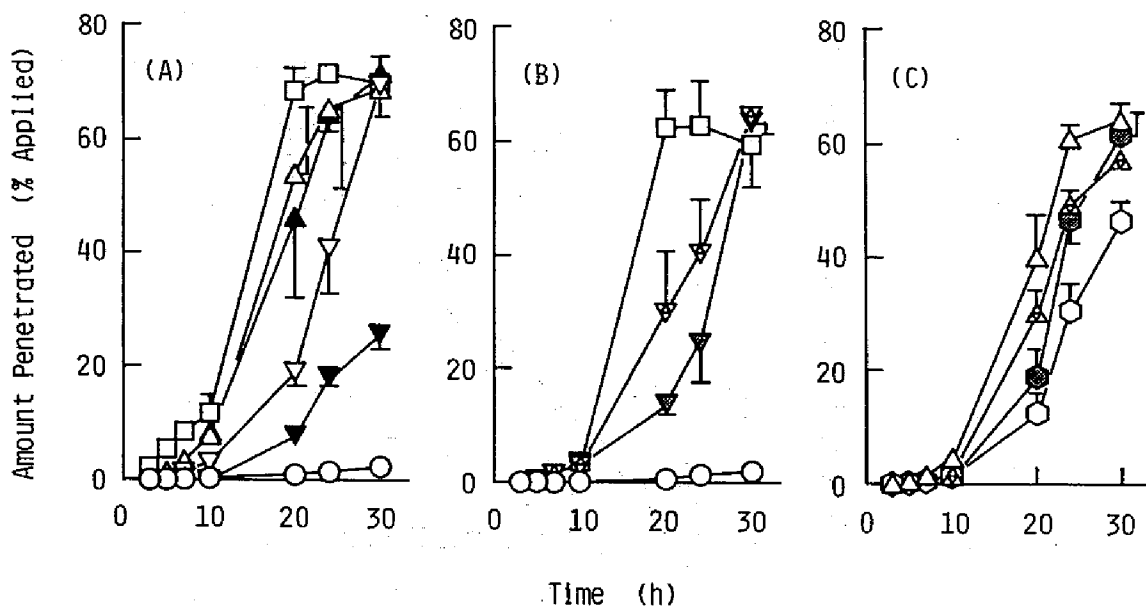


Figure 5. Penetration of MMC through rat skin at 37 °C. One milliliter of MMC suspensions (total concentration, 10 mM) in ethanol (○) or in 3.3 % ethanolic solutions of Azone (□), 7GU (▽), 7FU (△), 7GGU (⬡), 7GS (◀), 7FS (▲), 6GU (▼), 6GGU (⬢), 5GUDO (◆), or 5FU (♣) were applied. Each point represents the mean value of 3-7 experiments and vertical bars indicate standard errors of the mean. Two series of experiments were run; one of which is shown in (A) and the other is shown in (B) and (C).

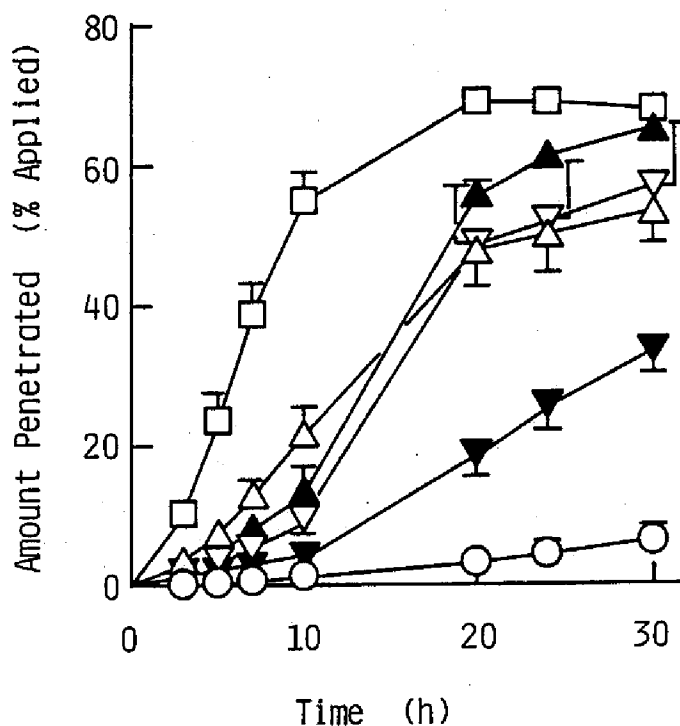


Figure 6. Penetration of MMC through rat skin at 37 °C. One milliliter of MMC suspension (total concentration, 10 mM) in ethanol was applied to the skin pretreated with ethanol or ethanolic solution of Azone (□), 7GU (▽), 7FU (△), 5GUD0 (◆), or 5FU (▲) for 24 h. Each point represents the mean value and vertical bars indicate standard errors of the mean.

Table II. Recovery Percentages of MMC from Donor and Receptor Medium after Diffusion Experiments with Hairless Mouse or Rat Skin^a

Skin	Enhancer	N	Drug Recovery (%)	
			Donor	Receptor
Hairless Mouse	Control	4	84.88 ± 1.68	3.53 ± 0.71
	Azone	3	2.06 ± 0.39	70.73 ± 4.35
	7GU	3	2.30 ± 0.24	76.47 ± 2.02
	7FU	3	5.23 ± 1.83	67.59 ± 1.87
	5GUDO	3	36.32 ± 6.16	39.59 ± 3.74
	5FU	3	2.83 ± 0.34	69.64 ± 0.64
Rat ^b	Control	4	77.00 ± 0.71	2.57 ± 0.87
	Control*	6	68.35 ± 4.39	1.98 ± 0.40
	Azone	4	0.93 ± 0.05	69.84 ± 1.22
	Azone*	3	0.93 ± 0.05	59.89 ± 7.23
	7GU	3	2.25 ± 0.85	69.37 ± 1.95
	7FU	4	1.87 ± 0.81	68.88 ± 4.87
	7FU*	5	1.21 ± 0.16	64.19 ± 3.83
	7GGU*	7	12.84 ± 2.94	46.71 ± 4.89
	7GS*	7	1.75 ± 0.54	64.83 ± 1.19
	7FS*	5	4.07 ± 0.63	57.59 ± 2.53
	6GU*	4	4.77 ± 1.72	63.38 ± 2.14
	6GGU*	4	6.15 ± 1.24	61.96 ± 3.88
	5GUDO	3	26.50 ± 0.75	25.95 ± 2.82
	5FU	4	1.77 ± 0.42	71.69 ± 2.98
	5FU	4	9.39 ± 1.72	64.52 ± 1.24
Rat (pre-treated)	Control	4	81.65 ± 2.96	6.45 ± 2.14
	Azone	4	3.09 ± 0.66	67.40 ± 1.11
	7GU	3	17.32 ± 5.44	56.70 ± 8.94
	7FU	4	13.15 ± 2.37	52.92 ± 4.54
	5GUDO	4	43.13 ± 5.10	33.26 ± 3.18

^a Data are expressed by means ± standard errors of the mean.

^b Two series of experiments were carried out. The second run is denoted by asterisk.

5GUDO also showed the smallest effect in this experimental system. The amount of MMC remaining in the donor in the control experiment was 81.7 %, which was reduced to 3.1 % by Azone and to about 10-17 % by the other enhancers except for 5GUDO (Table II).

1.2.4. Acute Skin Primary Irritancy of Enhancers

Figure 7 shows daily PII for erythema and edema induced by 24-h application of enhancer (100 %). In most cases, the maximum PII value was recorded in day 3-5, and in day 7 little erythema and edema were observed. The skin irritation by the polyprenylazacycloalkanone derivatives was less than that by Azone.

1.3. Discussion

To improve the percutaneous penetration of a drug, following approaches have been employed; (1) selection of a vehicle to increase thermodynamic activity or release rate of drug, (2) modification of drug molecules to have higher affinity for skin, and (3) use of percutaneous penetration enhancers to reduce the barrier properties of the skin.

Recently, the enhanced delivery of MMC through hairless mouse and rat skin was achieved by Sezaki et al. by means of chemical modification of MMC.^{19,20} Among various tested prodrugs of MMC, the most successful result was obtained with 1a-N-benzyloxycarbonylmitomycin C which penetrated the skin 5.3 times faster than MMC and was fully converted to MMC in the skin. In the present study, Azone, 7GU, 7FU, and 5FU enhanced MMC penetration about 40 times in hairless mouse skin and about 20 to 60 times in rat skin. Thus, employment of penetration enhancers appears to be more advantageous than the

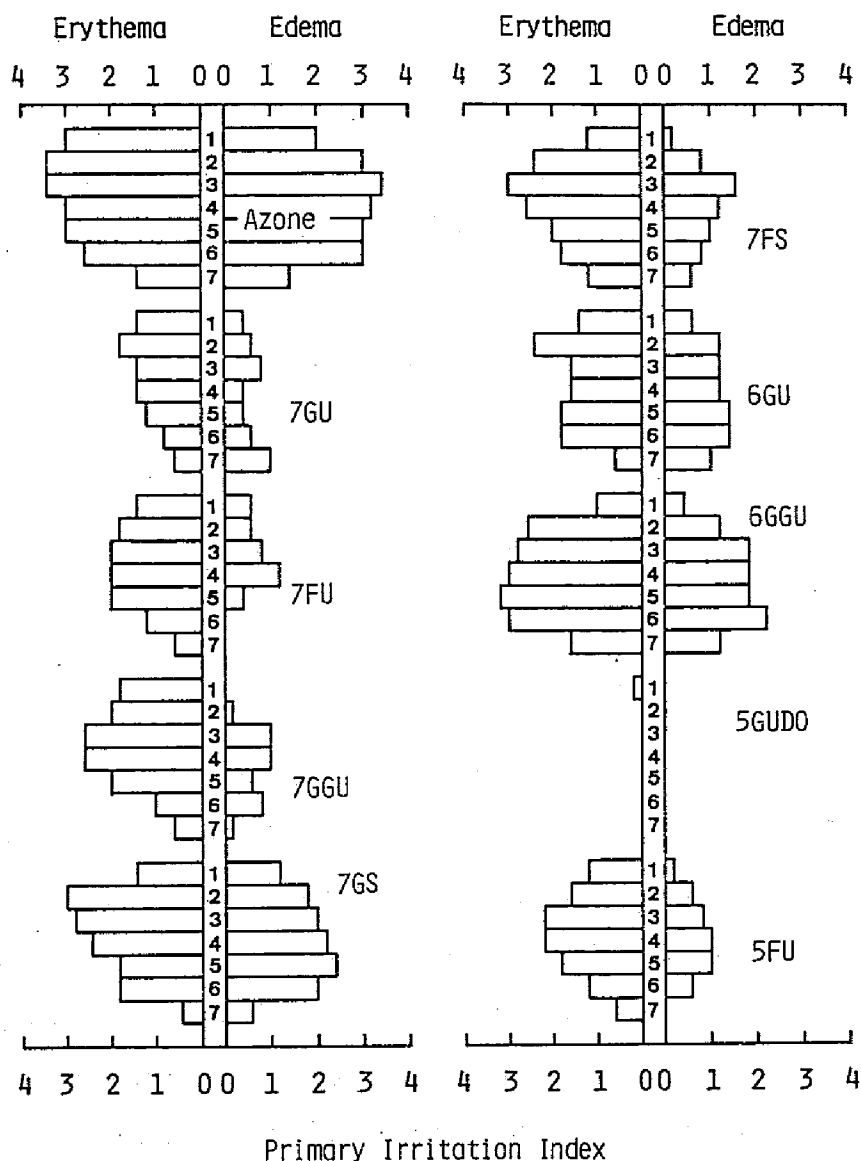


Figure 7. The primary irritation indices for enhancers in rabbit skin. The enhancers were applied, employing a Finn chamber, for 24 h without any dilution. Evaluations of erythema and edema were done for consecutive seven days (indicated in the mid-column) according to the Draize scoring system (maximum, 4),^{14,15} and averaged for five animals.

prodrug approach for MMC.

In general, the rate of permeation of the drug through the skin barrier is directly related to its thermodynamic activity above the barrier. This activity is maintained at the highest level as long as any solid material remains in the applied material, i.e. in the form of suspension.²¹⁾ The bending penetration profiles in the later stage of the diffusion experiment indicate that the maximum thermodynamic condition in the donor phase and sink condition in the receptor phase were not maintained any more at this stage. An apparent equilibrium in MMC concentration appeared to be reached between the donor phase, the skin, and the receptor phase at the end of the diffusion experiments employing the enhancers other than 5GUDO. Consequently, it is necessary to compare the effect of these enhancers in the early stage when the maximum thermodynamic activity condition of MMC in the donor phase and sink condition in the receptor phase were maintained.

7FU produced greater penetration of MMC than Azone or 7GU in hairless mouse skin at 10 h (Fig. 4), whereas Azone was more effective for rat skin (Fig. 5). The superior efficiency of Azone on rat skin was also demonstrated in the pretreatment experiments (Fig. 6). These results suggest that the effect of these enhancers might vary depending on the animal species to which they are applied. The site of action of Azone has been reported to be the stratum corneum.²²⁾ Variation in lipid and protein composition between both animal species may play an important role in this problem.

Barry reviewed the ideal properties of a penetration enhancer and suggested that the term such as penetration enhancers should be limited to materials which significantly enhance drug penetration through the epidermis but do not severely irritate or damage the skin.⁸⁾ At the first stage of developing a penetration enhancer, the toxicity problem of a candidate

compound should be examined as well as its penetration enhancing activity. The relatively low skin irritancy as well as excellent enhancing effect on the penetration of MMC proves that the polyprenylazacycloalkanone derivatives developed in this study are promising enhancers.

1.4. Summary

The effects of nine new percutaneous penetration enhancers containing an azacyclo ring and a polyprenyl chain on the percutaneous penetration of MMC were investigated in hairless mouse and rat skin. Based on the present results, it is concluded that some enhancers such as 7GU, 7FU, and 5FU have almost the equivalent potential to Azone. The penetration of MMC through rat skin was also increased by the pretreatment with them, suggesting that they had a direct effect on the skin.

The primary skin irritation was examined with rabbit dorsal skin in vivo. The polyprenylazacycloalkanone derivatives developed in this study induced less primary irritation (erythema and edema) than Azone.

These results suggested superior utilities of some of the tested polyprenylazacycloalkanone derivatives such as 7GU, 7FU, and 5FU as percutaneous penetration enhancers.

Chapter 2. Relationship between Structure and Activity of Percutaneous Penetration Enhancer

In Chapter 1, the phenomenal effects of nine polyprenylazacycloalkanone derivatives on the penetration of mitomycin C (MMC) through hairless mouse and rat skin were examined as well as their skin irritancy. In order to establish the strategy for designing percutaneous penetration enhancer, we should get enough information on structure-activity relationship of enhancers. In this chapter, further examination was carried out on this problem using a model drug, 6-mercaptopurine (6-MP), by the in vitro diffusion experiment with guinea pig skin.¹¹⁾ The experimental system employed in this chapter makes it possible to discuss the action of the enhancers more precisely because of; 1) employment of flow-through type diffusion cell offers satisfactory sink condition in the receptor phase and many consecutive samplings which support a mathematical analysis of diffusion process, 2) pretreatment dosing of enhancers gives direct effect on skin, 3) one guinea pig affords plural skin samples which diminishes individual difference problem, 4) 6-MP avoids the problem relating metabolic decomposition, 5) employment of radioisotope analysis enables exact determination of drug amounts in receptor, donor, and skin.

In a diffusion experiment, the analysis of data based on Fick's first or second law of diffusion gives useful parameters such as diffusion constant and partition coefficient of the drug between skin and vehicle.^{23,24)} In this chapter, a diffusion model considering the donor concentration decrease (finite dose system) was constructed and the Laplace transforms corresponding to the amounts of drug in the receptor solution, in the skin, and in the donor solution were derived based on this model. The computer analysis of the

penetration profiles based on this diffusion model gave two parameters corresponding to drug diffusion and partitioning and enabled us to discuss the mechanism of action of the penetration enhancers and their structure-activity relationship in physicochemical terms. The mean transit time (MTT) for drug transport through the skin barrier calculated from these two parameters was shown to be a good index of drug penetrability.

2.1. Experimental

2.1.1. Materials

6-MP and [^{14}C]6-MP (65 mCi/mmol) were obtained from Nacalai Tesque Inc., Japan and Commissariat A L'Energie Atomique, France, respectively. 1-Dodecylazacycloheptan-2-one (Azone) was kindly supplied by Nelson Research Center, USA. 1-Geranylazacycloheptan-2-one (7GU), 1-farnesylazacycloheptan-2-one (7FU), 1-geranylgeranylazacycloheptan-2-one (7GGU), 1-(3,7-dimethyloctyl)azacycloheptan-2-one (7GS), 1-(3,7,11-trimethyldodecyl)azacycloheptan-2-one (7FS), 1-geranylazacyclohexan-2-one (6GU), 1-geranylgeranylazacyclohexan-2-one (6GGU), 1-geranylazacyclopentan-2,5-dione (5GUDO), and 1-farnesylazacyclopentan-2-one (5FU) were synthesized by Kuraray Co., Japan. Other chemicals used were of analytical grade.

2.1.2. In Vitro Percutaneous Penetration Experiment

Through all the experiments, the full thickness of skin obtained from the dorsal surface of a male guinea pig (Hartley strain, ca. 250 g) immediately prior to the experiment was used. After the removal of the hair, with electric clippers, and adipose tissue, the skin was punched out into a 3-cm diameter disk and mounted on a flow-through type diffusion cell with dermis

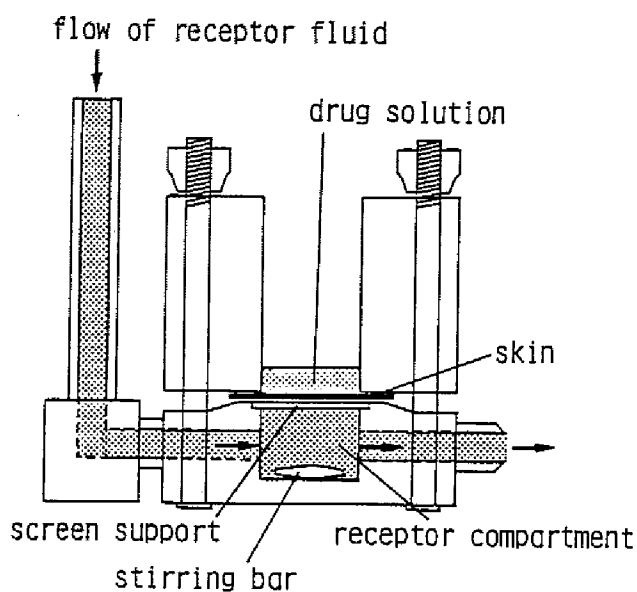


Figure 8. Flow-through type diffusion cell.

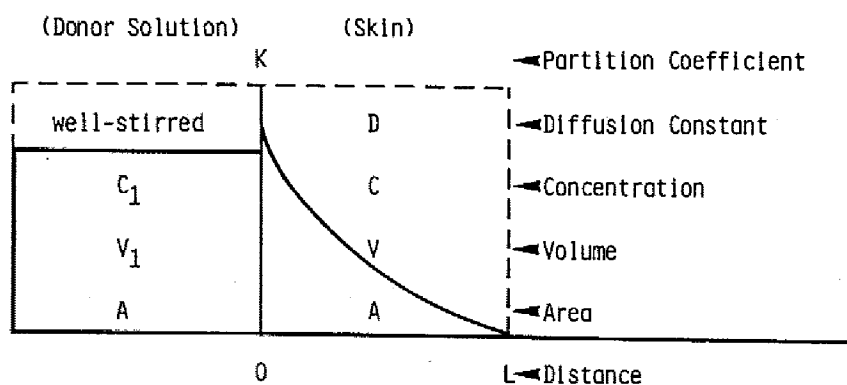


Figure 9. The one-layer skin model for the finite dose system. This model assumes a well-stirred condition in the donor solution and a perfect skin condition in the receptor phase.

side facing to the receptor cell (exposed area, 3.14 cm^2) (Fig. 8).^{25,26}) Four pieces of skin were obtained from each animal. The apparatus was thermostated at 37°C in a water bath throughout the experiment.

To examine the direct effects of enhancers on skin, the skin mounted on a diffusion cell was pretreated with 0.2 ml of a 0.1 M ethanolic solution of an enhancer at 37°C . Twenty-four hours later, ethanol in the donor cell was evaporated with hair dryers (three periods of 1 min of drying with 3-min intervals for each cell). It took 15 min to accomplish this operation. At zero time of the diffusion experiment, a 1-ml aliquot of a 1 mM aqueous solution of 6-MP, containing about 0.4 μCi of $[^{14}\text{C}]6\text{-MP}$, was applied to each donor cell.

The dermal side of the skin was continuously washed with saline containing streptomycin sulfate (50 mg/L, Sigma Chemical Co., USA) and penicillin G potassium salt (30 mg/L, gift from Toyo Jozo, Japan) which flowed at about 6 ml/h. The receptor fluid was collected at every 90 min for 24 h. Throughout the experiment, the donor cell was capped with a silicon stopper to prevent the donor solution from evaporation.

At the end of the 24-h experiment, 6-MP remaining in the donor cell was recovered with 8 ml of water. The skin was removed from the diffusion cell and a 1-cm diameter disk was punched out for the determination of 6-MP accumulation in the skin.

The determination of $[^{14}\text{C}]6\text{-MP}$ was carried out by liquid scintillation counting (Model LSC-903, Aloka). One milliliter of each aqueous sample was added to a vial with 5 ml of scintillation cocktail (Univer-Gel II; Nacalai Tesque, Inc., Japan). Each skin sample was dissolved in 1 ml of Soluene-350 (Packard Instrument, USA) and 5 ml of scintillation cocktail was added.

The amount of 6-MP appearing in the receptor fluid for 24 h and that

accumulating in the skin for 24 h were compared between the 12 experimental groups ($n = 3-5$) by means of a one way ANOVA, followed by Fisher's t-test.

2.2. Theoretical

It is defined in this chapter that A is the effective diffusion area, C is the drug concentration (C_0 is zero time concentration in the donor), D is the diffusion constant in skin, K is the partition coefficient between skin and donor solution, L is the effective length of diffusion through skin, s is the Laplace variable with respect to time t , V is the volume (product of A and L), x is the distance, and X_0 is the applied dose. Subscripts 1 and 2 stand for vehicle and skin, respectively. Parameter d is defined as:

$$d = L \cdot (s/D)^{1/2} \quad (2)$$

In the experimental system treated in this chapter, the drug concentration in the donor phase decreases with time due to its penetration through the skin (finite dose system). A perfect sink condition in the receptor phase should be established because this phase is continuously washed by saline. A diffusion model shown in Fig. 9 was constructed for analyzing the diffusion process assuming a well-stirred condition in the donor and regarding the skin as a plane barrier membrane (one-layer skin model).²⁷⁾ Fick's second law of diffusion is:

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad (3)$$

The initial and boundary conditions for this model are (C_1 is the donor concentration):

$$C = 0 \quad (t = 0, 0 < x \leq L) \quad (4)$$

$$C_1 = C_0 \quad \text{or} \quad C_1 V_1 = X_0 \quad (t = 0, -V_1/A \leq x \leq 0) \quad (5)$$

$$V_1 \frac{d}{dt} C_1 = \frac{V_1}{K} \cdot \frac{d}{dt} C = DA \left(\frac{\partial C}{\partial x} \right)_{x=0} \quad (\text{at } x = 0) \quad (6)$$

$$C = 0 \quad (\text{at } x = L) \quad (7)$$

According to this model, the Laplace transforms for the total amounts of drug in the receptor (\bar{Q}), the donor (\bar{X}), and the skin (\bar{M}) are expressed as (see Appendix 1):

$$\bar{Q} = KV_2 X_0 / s / g(s) \quad (8)$$

$$\bar{X} = V_1 \cdot d \cdot \sinh(d) \cdot X_0 / s / g(s) \quad (9)$$

$$\bar{M} = KV_2 X_0 \cdot [\cosh(d) - 1] / s / g(s) \quad (10)$$

where,

$$g(s) = V_1 \cdot d \cdot \sinh(d) + KV_2 \cosh(d) \quad (11)$$

The penetration profiles of 6-MP were fitted to Eq. 8 using MULTI(FILT),²⁸⁾ which is a nonlinear least squares computer program based on fast inverse Laplace transform algorithm. This program is written in MS-FORTRAN and run on personal computer PC-9801 VX, NEC, Japan, to obtain the diffusion parameter (D') and partition parameter (K'). Since it is difficult to correctly determine the real diffusion barrier thickness, these two parameters involving skin thickness are defined.

$$D' = D/L^2 \quad (12)$$

$$K' = K \cdot L \quad (13)$$

In Eqs. 8-10, KV_2 is replaced by $K'A$ to obtain the K' value ($A = 3.14 \text{ cm}^2$).

The mean transit time (MTT) of 6-MP from the donor solution to the

receptor was calculated by the following equation ($V_1 = 1$ ml) (see Appendix 1): 29,30)

$$MTT = \frac{1}{2D'} + \frac{V_1}{D'K'A} \quad (14)$$

2.3. Results

2.3.1. Relationship between Structure and Activity of Enhancer

Figures 10A-C show the penetration profiles of 6-MP through guinea pig skin which was pretreated with enhancers. The amounts of 6-MP recovered in the donor and receptor phases and the skin at the end of each diffusion experiment are summarized in Table III.

Two control experiments were run in this study. In one, 6-MP solution was applied to guinea pig skin pretreated with ethanol (open circle in Figs. 10A-C), and in another, 6-MP solution was applied to guinea pig skin without any pretreatment (closed circle in Fig. 10B). Pretreatment with ethanol enhanced 6-MP penetration by three times, (Table III). As shown in Figs. 10A-C, all tested enhancers except for 5GUDO showed a significant increase in 6-MP penetration compared with pure ethanol, under the same pretreatment condition.

The increasing ratio of 6-MP penetration provoked by enhancers compared with that in ethanol pretreated control is plotted against the number of tail-chain carbons of each enhancers (Fig. 11A).

In the three compounds with the azacycloheptan-2-one ring, namely, 7GU (C10 tail chain), 7FU (C15 tail chain), and 7GGU (C20 tail chain), the compound with shorter tail chain (7GU) showed the greater enhancement of 6-MP

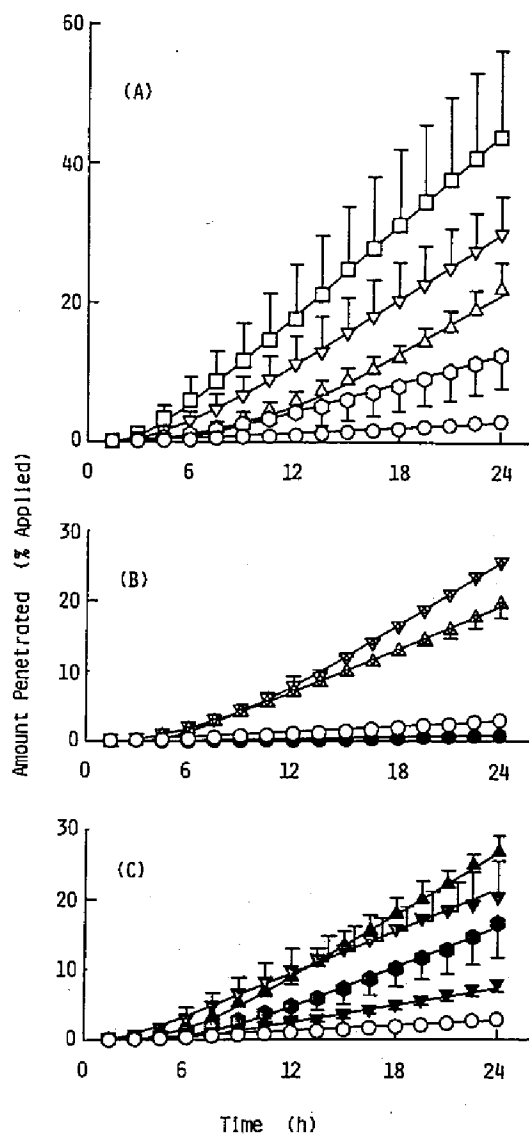


Figure 10. Penetration of 6-MP through guinea pig skin pretreated with $20 \mu\text{mol}/3.14 \text{ cm}^2$ of Azone (□), 7GU (▽), 7FU (△), 7GGU (◻), 7GS (▽), 7FS (▲), 6GU (▽), 6GGU (◻), 5GUDO (▼), or 5FU (▲). Key: (○) ethanol pretreated control and (●) nonpretreated control. Each point represents the mean value of 3–5 experiments and vertical bars indicate standard deviations. The connected lines were obtained by computer fitting of data to Eq. 8.

Table III. Recovery Percentages of 6-MP after 24-Hour Diffusion Experiments with Guinea Pig Skin^a

Enhancer	N	Drug Recovery (%)			
		Total	Donor	Skin ^d	Receptor ^e
C(NP) ^b	5	93.73 ± 5.41	87.68 ± 5.48	5.23 ± 0.39	0.83 ± 0.57
C(P) ^c	3	100.89 ± 3.57	91.24 ± 3.86	6.69 ± 2.07	2.96 ± 1.63
Azone	4	96.68 ± 2.82	33.43 ± 2.65	19.36 ± 9.95	43.89 ± 12.55
7GU	3	108.26 ± 2.33	46.87 ± 5.40	31.77 ± 2.14	29.63 ± 5.56
7FU	3	103.79 ± 3.29	56.43 ± 4.01	25.05 ± 5.85	22.30 ± 3.44
7GGU	5	101.97 ± 6.44	72.88 ± 4.66	16.59 ± 4.95	12.49 ± 4.85
7GS	3	100.08 ± 4.83	42.34 ± 5.64	32.37 ± 2.13	25.37 ± 0.99
7FS	3	104.22 ± 5.13	62.08 ± 2.78	22.15 ± 5.94	19.99 ± 2.44
6GU	3	103.82 ± 4.80	53.72 ± 5.59	29.96 ± 6.47	20.14 ± 5.39
6GGU	3	102.23 ± 5.16	59.69 ± 10.68	25.88 ± 2.35	16.66 ± 5.01
5GUDO	3	105.38 ± 3.84	80.33 ± 3.09	17.33 ± 5.45	7.72 ± 0.85
5FU	3	102.29 ± 4.54	45.41 ± 5.12	29.68 ± 7.77	27.20 ± 1.97

^a Data are expressed by means ± standard deviations.

^b Control experiment with guinea pig skin without pretreatment.

^c Control experiment with guinea pig skin pretreated with ethanol.

^d Statistical significance (Fisher's t-test, $p < 0.05$) was recognized between the groups (C(NP) < Azone, 7GU, 7FU, 7GGU, 7GS, 7FS, 6GU, 6GGU, 5GUDO, 5FU), (C(P) < Azone, 7GU, 7FU, 7GGU, 7GS, 7FS, 6GU, 6GGU, 5GUDO, 5FU), (Azone < 7GU, 7GS, 6GU, 5FU), (7GU > 7GGU, 7FS, 5GUDO), (7FU > 7GGU), (7GGU < 7GS, 6GU, 6GGU, 5FU), (7GS > 7FS, 5GUDO), (6GU > 5GUDO), and (5GUDO < 5FU).

^e Statistical significance (Fisher's t-test, $p < 0.05$) was recognized between the groups (C(NP) < Azone, 7GU, 7FU, 7GGU, 7GS, 7FS, 6GU, 6GGU, 5FU), (C(P) < Azone, 7GU, 7FU, 7GGU, 7GS, 7FS, 6GU, 6GGU, 5FU), (Azone > 7GU, 7FU, 7GGU, 7GS, 7FS, 6GU, 6GGU, 5GUDO, 5FU), (7GU > 7GGU, 7FS, 6GU, 6GGU, 5GUDO), (7FU > 7GGU, 5GUDO), (7GGU < 7GS, 5FU), (7GS, 7FS, 6GU, 6GGU > 5GUDO), and (6GGU, 5GUDO < 5FU).

penetration. Comparison of the enhancers with a six-member ring (6GU and 6GGU) showed greater enhancement with a shorter alkenyl chain (6GU), while the penetration profile suggests a plateau in the activity of 6GU at the later stage of experiment (Fig. 10C).

A significant difference between a branched alkyl chain (7GS and 7FS) was not observed (Table III). Less enhancement was found for compounds with a C20 chain and 6GGU. Less enhancing activity of these compounds was also demonstrated for the penetration of MMC (Figs. 5A-C in Chapter 1).

The hydrogenation of the alkenyl chain, which gives 7GS and 7FS from 7GU and 7FU, respectively, seemed to have no effect on the enhancing power. 7FS, a methylated derivative of Azone in three positions of its dodecyl chain, yielded half the enhancement by Azone.

The size of the azacyclo ring had less effect on the enhancing activity than did the tail chain length. In the enhancers with a geranyl chain, the enhancer with a seven-member ring (7GU) was significantly more effective than the one with six-member ring (6GU). In the case of enhancers with a farnesyl or geranylgeranyl chain, no significant difference was observed (7FU vs 5FU and 7GGU vs 6GGU).

The number of carbonyl groups greatly affected the enhancing activity. 5GUDO, which has two carbonyl groups in its azacyclopentane ring, was far less effective than the other enhancers. The importance of the number of carbonyl group for the enhancing activity was also shown when it was administrated with the penetrant, MMC (Fig. 5A).

The 6-MP accumulation in skin was also increased by pretreatment with penetration enhancers (Table III). This amount was significantly greater after application of enhancers 7GU and 7GS compared with Azone, 7GGU, 7FS, or 5GUDO. Figure 11B shows the relationship between the tail chain length of

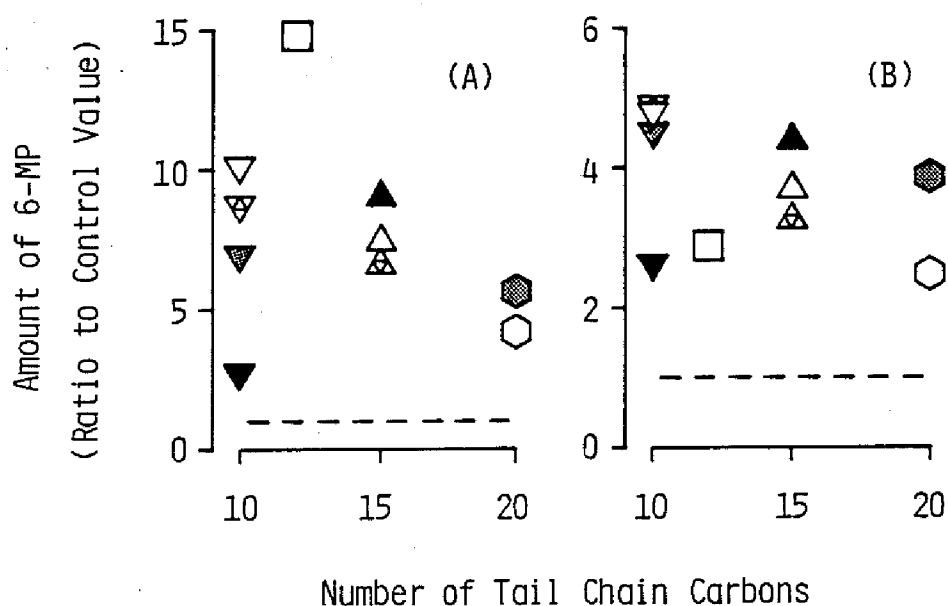


Figure 11. The relationships between the number of tail chain carbons and drug amounts recovered in the receptor (A) or in the skin (B). Values are expressed as the ratios to each control value. Dotted line indicates the control level. Key: (\square) Azone, (∇) 7GU, (\triangle) 7FU, (\hexagon) 7GGU, (∇ with dot) 7GS, (\triangle with dot) 7FS, (∇) 6GU, (\hexagon with dot) 6GGU, (∇) 5GUDO, and (\blacktriangle) 5FU.

enhancers and increase in the skin accumulation of 6-MP. As seen in the case of 6-MP penetration, the enhancers with a shorter tail chain had a superior effect. Figure 12 shows the relationship between the amounts of 6-MP in skin and those in the receptor phase at the end of diffusion experiments for each enhancer. A relatively good correlation was found between increased skin accumulation and the penetration of 6-MP for all enhancers except for Azone. In the case of Azone, the enhancement of the penetration of 6-MP was the largest but the increment of the 6-MP skin accumulation was significantly less than 7GU, 7GS, 6GU, and 5FU.

2.3.2. Effect of Pretreatment with Enhancers on Diffusion and Partition Parameters Obtained Based on One-Layer Skin Model

The penetration profiles were fitted to Eq. 8 by nonlinear least squares computer program (MULTI(FILT)), and parameters for percutaneous penetration of 6-MP were calculated (Table IV).

Pretreatment of skin with ethanol increased the diffusivity of 6-MP in skin (D') by a factor of three over the nonpretreated control. The K' value for ethanol pretreated skin was two-thirds that of nonpretreated skin, suggesting that the ethanol pretreatment slightly decreases the 6-MP partitioning to skin. The increased penetration of 6-MP caused by ethanol pretreatment was mainly due to the increase in the D' value.

Pretreatment with an enhancer solution also increased D' values compared with the nonpretreated control but the degree of increment was almost equal to or less than that induced by the ethanol pretreatment (Fig. 13B). This suggests that an increase in diffusivity of 6-MP through skin is not the determinant of enhancing activity examined in the present study. 6GU caused somewhat large D' value compared with the other enhancers. The large D'

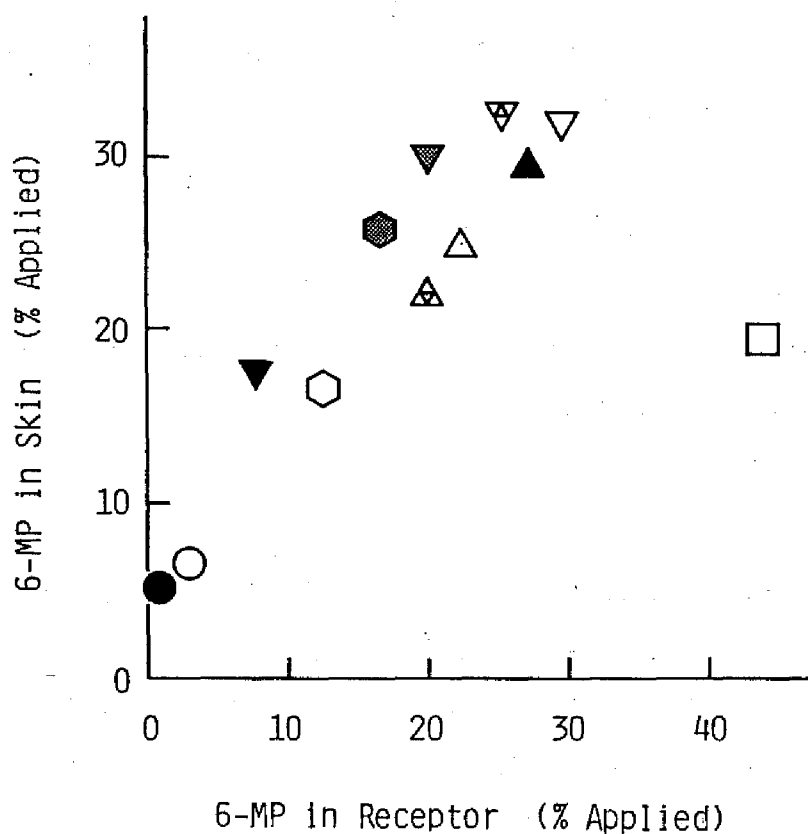


Figure 12. The relationship between the amounts of 6-MP recovered in the receptor compartment and in the skin after 24-h diffusion experiments. Key: (●) control with nonpretreated skin, (○) control with ethanol pretreated skin, (□) Azone, (▽) 7GU, (△) 7FU, (⬡) 7GGU, (▽) 7GS, (△) 7FS, (▽) 6GU, (⬢) 6GGU, (▼) 5GUDO, and (▲) 5FU.

Table IV. Diffusion Parameter (D'), Partition Parameter (K'), and Mean Transit Time (MTT) for Percutaneous Penetration of 6-MP^a

Enhancer	$D' \times 10^3 \text{ (h}^{-1}\text{)}$	$K' \times 10^3 \text{ (cm)}$	MTT (h)
C(NP)	12.01	18.72	1457
C(P)	35.15	12.75	724.5
Azone	27.63	557.5	38.76
7GU	23.94	359.1	57.91
7FU	15.25	532.0	72.02
7GGU	24.82	104.4	143.0
7GS	16.27	625.8	61.99
7FS	27.28	161.3	90.67
6GU	51.01	74.57	93.48
6GGU	17.83	257.1	97.48
5GUDO	19.20	90.18	209.9
5FU	21.12	400.2	61.33

^a C(NP) and C(P) stand for the nonpretreated and ethanol pretreated controls, respectively. Parameters were obtained by the computer fitting of the penetration profile to Eq. 8. The total recovery percentage in Table III was employed as the dose (X_0) in Eq. 8.

value in 6GU may be due to a somewhat anomalous penetration profile (Fig. 10C) in which the rate of 6-MP penetration seems to level off in the later stage of the penetration. From such a profile, a large D' value should be calculated.

The K' value, corresponding to the degree of partitioning of 6-MP to skin surface, was drastically increased by pretreatment with an enhancer. Azone, 7FU, and 7GS increased this value more than 7GGU, 7FS, 6GU, and 5GUDO, and 5FU did more than 7GGU, 6GU, and 5GUDO. Fig. 13A shows that enhancers with a shorter tail chain increase the partition parameter more than ones with longer chain (the small value for 6GU is due to an anomalous penetration profile).

The time course of drug amounts in the donor solution and skin can be estimated based on the one-layer skin model by Eqs. 9 and 10, respectively, using the partition parameter and diffusion parameter obtained by Eq. 8. A typical pattern is shown in Fig. 14 for 6-MP penetration through the skin pretreated with 5FU. The estimated profiles for drug amounts in the donor, skin, and receptor accorded well with the observed values. Figure 15A shows the relationship between the amounts of 6-MP in the donor at the end of 24-h experiment and those estimated using the obtained parameters based on the one-layer skin model (Eq. 8). The same relationship is described in Fig. 15B for the amount of 6-MP in the skin. Good correlations were observed between experimental and estimated values. This justifies the analysis based on the one-layer skin model in the present experimental system.

The MTT for the 6-MP skin penetration, calculated according to Eq. 14, shows a hyperbolic relationship with the cumulative amount of 6-MP appearing in the receptor (Fig. 16A). The reciprocal of MTT, which has a dimension of first-order velocity constant, shows good correlation with the amount of 6-MP in the receptor (Fig. 16B). The plots of total penetrating amounts of 6-MP versus K_p values are more scattered (data not shown).

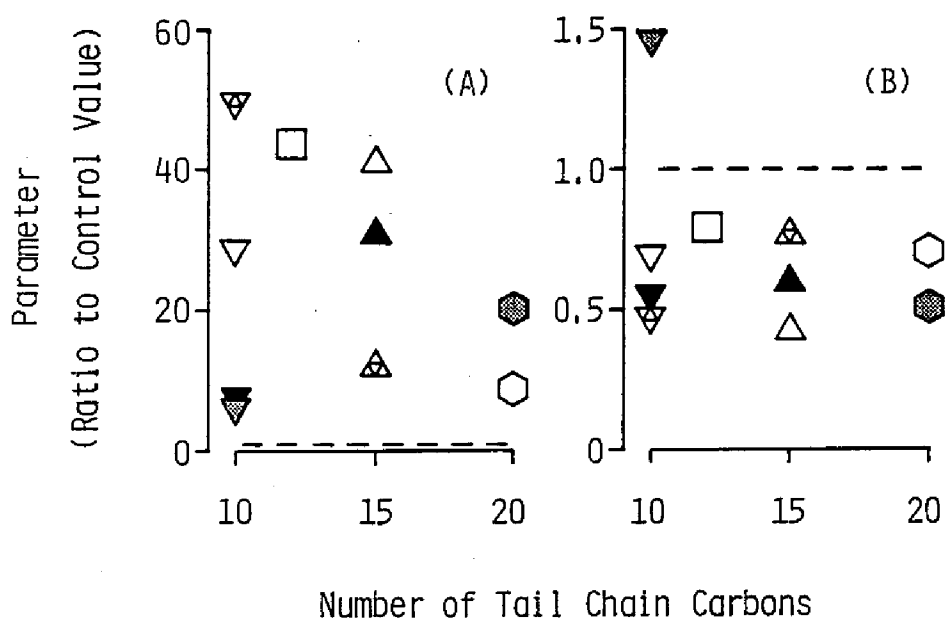


Figure 13. The relationships between the number of tail chain carbons and the partition parameter (A) or diffusion parameter (B) for 6-MP penetration. Values are expressed as the ratios to each control value. Dotted line indicates the control level. Key: (□) Azone, (▽) 7GU, (△) 7FU, (⬡) 7GGU, (▽) 7GS, (⬢) 7FS, (▽) 6GU, (⬢) 6GGU, (▼) 5GUDO, and (▲) 5FU.

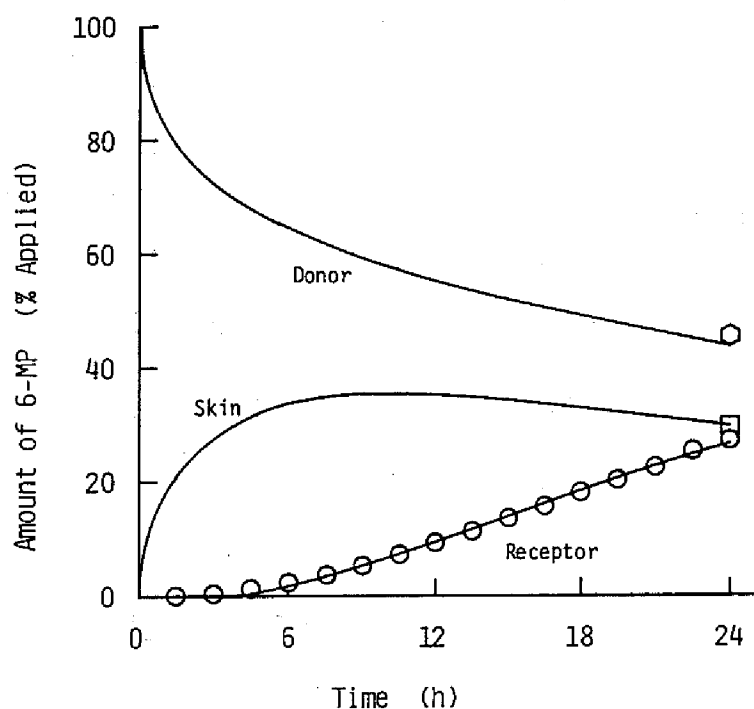


Figure 14. Simulated profiles of 6-MP penetration through guinea pig skin pretreated with 5FU. Symbols represent the observed values. The continuous line for receptor is obtained by computer fitting of penetration data to Eq. 8 and those for donor and skin are the simulated profiles by Eqs. 9 and 10 for the calculated parameters.

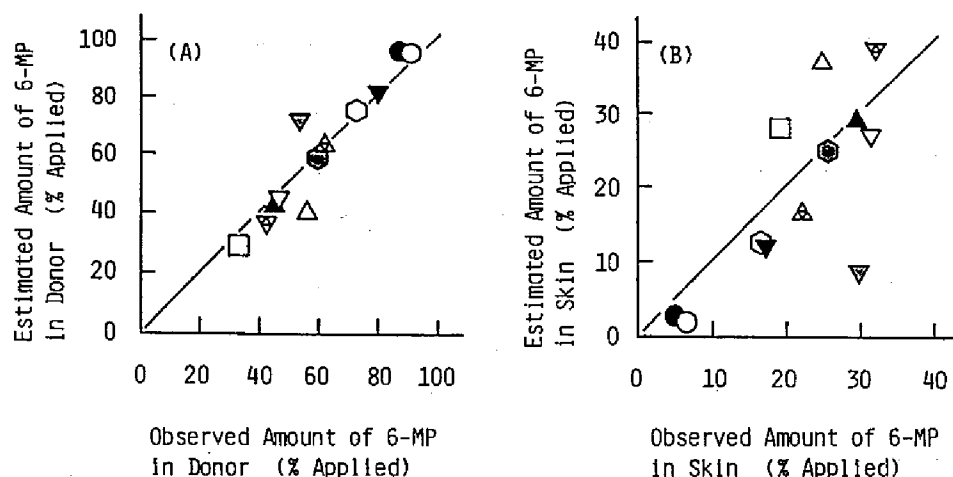


Figure 15. The relationships between estimated and observed amounts of 6-MP in the donor solution (A) or in the skin (B). Calculations were carried according to Eqs. 9 and 10 using the determined diffusion parameter and partition parameter listed in Table IV. The lines are given to show the unit slope. Key: (●) nonpretreated control, (○) ethanol pretreated control, (□) Azone, (▽) 7GU, (△) 7FU, (◻) 7GGU, (▽) 7GS, (△) 7FS, (▽) 6GU, (◻) 6GGU, (▽) 5GUDO, and (▲) 5FU.

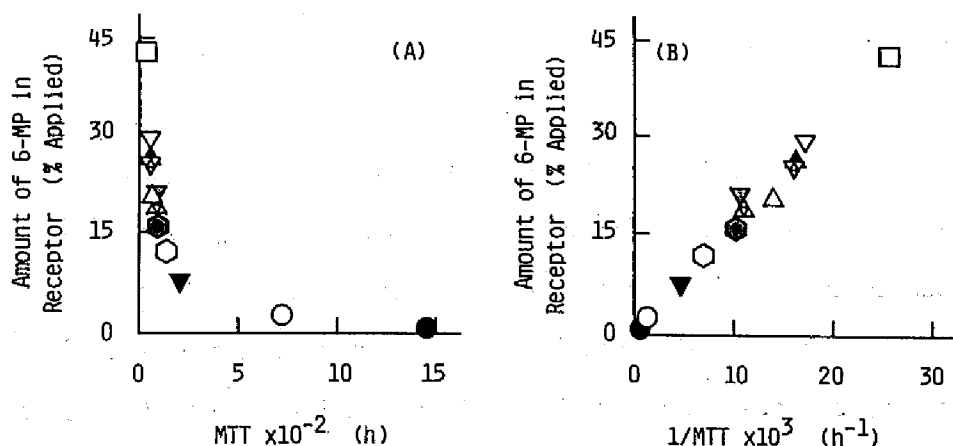


Figure 16. The relationships between mean transit time (A) or its reciprocal (B) and the total amount of 6-MP appearing in the receptor. Key: (●) nonpretreated control, (○) ethanol pretreated control, (□) Azone, (▽) 7GU, (△) 7FU, (◻) 7GGU, (▽) 7GS, (△) 7FS, (▽) 6GU, (◻) 6GGU, (▽) 5GUDO, and (▲) 5FU.

2.3.3. Relationship between Structure and Skin Irritancy of Enhancers

The primary irritation indices (Fig. 7) on both erythema and edema were averaged for seven days and are plotted against the reciprocal of MTT value for all enhancers in Fig. 17. Azone had the greatest enhancing activity, but irritated skin the most. 5GUDO had almost no irritancy, but the activity as an enhancer was also the least. In the enhancers with an unsaturated side chain, those with a geranylgeranyl chain gave the severest irritation to rabbit skin (7GGU > 7FU > 7GU and 6GGU > 6GU). The enhancers with an alkenyl chain had less cutaneous irritancy compared with those with an alkyl chain (7GU > 7GS and 7FU > 7FS). In enhancers with an unsaturated chain, those with a six-member ring seemed to have greater irritancy (6GGU > 6GU > the other enhancers with alkenyl chain). From the balance of the penetration enhancing activity and the irritation inducing activity, the enhancers with geranyl or farnesyl chains such as 7GU, 7FU, and 5FU are concluded to be the favorable ones.

2.4. Discussion

Pretreatment with ethanol enhanced the 6-MP penetration threefold. Pretreatment of skin for 24 h in vitro seems to hydrate skin and enhance the penetration of 6-MP because of continuous washing of the dermis side of skin. The hydration of skin results in the increased diffusivity.³¹⁻³³⁾ In this study, the elevation was observed in D' but not in K' (Table IV). The alteration of skin permeability by pure ethanol was reported previously.³⁴⁾ Scheuplein and Ross reported the extraction of stratum corneum lipid by ethanol. The delipidized skin showed a great increase in permeability and decrease in activation energy for water diffusion.³⁵⁾ Such an effect of

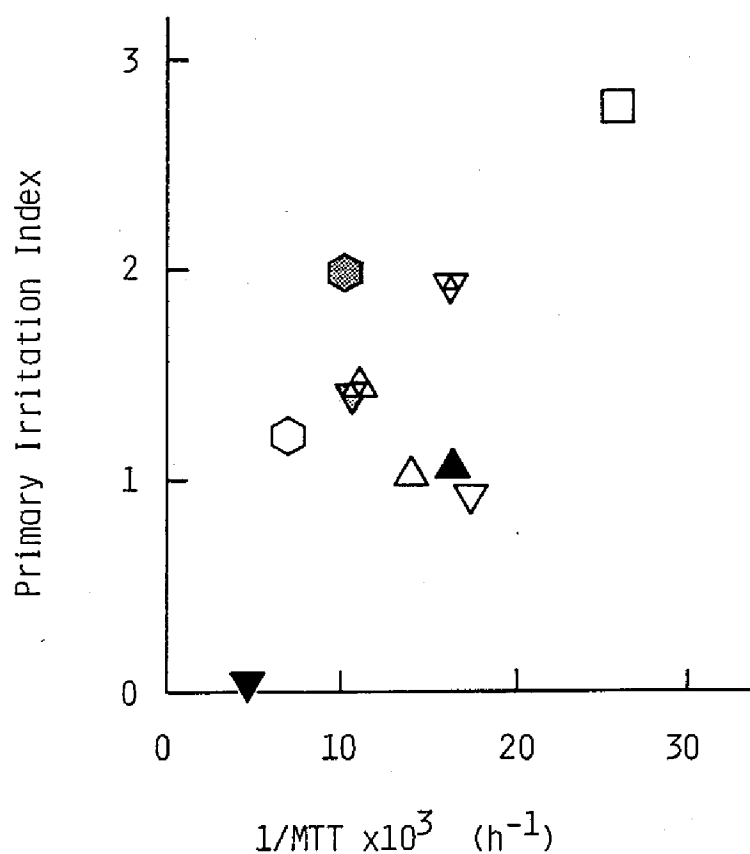


Figure 17. The relationship between enhancing effect on the 6-MP penetration and skin irritancy of enhancers. Primary irritation indices (Fig. 7) were averaged for seven days. Key: (\square) Azone, (∇) 7GU, (\triangle) 7FU, (\circ) 7GGU, (∇) 7GS, (\triangle) 7FS, (∇) 6GU, (\circ) 6GGU, (∇) 5GUDO, and (\blacktriangle) 5FU.

ethanol, if it exists, may also lead to an increase in D' value.

From the literature, various information was obtained regarding the effects of chemical structure on the potency of penetration enhancers. An optimal chain length has been reported for many enhancers, such as alkylmethyl sulfoxides,³⁶⁾ fatty acids, fatty alcohols,^{37,38)} and surfactants.³⁹⁾ From these reports, the optimal tail chain length of an enhancer seems to be about C12, regardless of the series of compounds. The enhancers having a polyprenyl chain (with trans double bonds and branched methyl groups) also seem to have optimum number of tail-chain carbons at C10 or C15.

The enhancing effects of cis-unsaturated C18 acids and alcohols were reported to increase with an increase in the number of double bonds.³⁷⁾ In the present study, the unsaturated tail chain has trans double bonds, and enhancing effect was nearly equivalent to a saturated tail chain. Karnovsky reported that the cis-unsaturated fatty acids disordered the lipid interior while the trans-unsaturated and saturated ones did not.⁴⁰⁾ Results obtained from IR and DSC measurements showed increased lipid fluidity following treatment of the stratum corneum with the cis-monoenoic acids, while the corresponding trans acids showed relatively little effect.⁴¹⁾ The trans-form double bond seems to have little effect on the enhancing activities.

The C12 diacids, dodecanedioic acid and trans-dodecenedioic acid, were reported not to appreciably increase naloxone flux, while the C12 mono acid, lauric acid, showed large enhancement.³⁷⁾ In this investigation, the enhancer with two carbonyl groups (5GUDO) showed the lowest activity. The number of polar group seems to be a determinant of enhancing power.

Information relating chemical structure and skin irritancy is available in the literature. Surfactants such as sodium alkylsulfates or fatty acids and fatty alcohols were reported to have maximum irritancy in their homologues

with a C12 alkyl chain.⁴²⁻⁴⁶⁾ In the polyprenylazacycloalkanone derivatives tested in this study, the largest irritating activity was observed in enhancers with a C20 tail chain. The existence of trans double bonds and/or branched methyl groups may be one of the reasons for these different results. Reller reported that compounds with double bonds were more irritative than those with a saturated alkyl chain (the double bonds are thought to be cis form because natural compounds were used).⁴⁶⁾ However the enhancers having a terpene chain, which contain trans double bonds, are less skin irritating than those having alkyl chain. The little effect of trans-unsaturated compounds on lipid interior or fluidity^{40,41)} seems to lead to the less skin irritancy of the present compounds.

Tables III and IV suggest the linear dependence of 6-MP skin accumulation on the K' value, independent of D' value. Increase in both K and L values can be considered for the increase in K' value. The effect of L may be little, if any, because it is hardly possible that skin barrier thickness increases by a factor of 10-20. Solvation or hydration may increase the L value,³⁴⁾ however, that degree may not be so different between enhancers. Also if L could be a determinant, the D' (D/L^2) value may decrease according to the increase of the K' value, which is not observed (Figs. 13A and B). The enhancers increase the drug partitioning into skin and enhance its penetration due to the increased concentration gradient in the skin barrier. The enhancers in skin appear to modify the nature of skin barrier to be a "good solvent" for a drug and increase 6-MP solubility.

The one-layer skin model assumes a well-stirred condition in the donor solution. This assumption is acceptable when the diffusion constant (more precisely, diffusion parameter, D/L^2) of a drug in the donor solvent is sufficiently large. In this experiment, water was employed as the vehicle

for 6-MP and the well-stirred condition seemed to be maintained.

There are some reports based on percutaneous absorption models that consider drug diffusion in the skin.^{29,30,47,48} The method using MULTI(FILT) is easier to deal with than others to extract parameters for percutaneous penetration, such as the diffusion constant or partition coefficient from the experimental data.

The permeability constant ($K_p = DK/L$) is commonly used as an index for drug penetrability. However, K_p relates only to the penetration rate at steady state and does not include any information on the lag phase. A drug with a large diffusion constant and a small partition coefficient may penetrate in larger amounts than a drug with a small diffusion constant and a large partition coefficient, even if their permeability constants are the same.

In this study, the MTT for the drug penetration through the skin was obtained by Eq. 14 according to the one-layer skin model. Using the relationship, LT (lag time) = $1/6D'$,⁴⁹ which is derived from diffusion model for the infinite dose system, Eq. 14 can be expediently rewritten as:

$$MTT = 3LT + \frac{V_1}{K_p \cdot A} \quad (15)$$

MTT is an index which considers not only the apparent steady state penetration rate (presented by K_p) but also the lag phase (presented by LT). The drug absorption is faster when the applied volume is small and the applied area is large.

Equation 14 is basically similar to the equation for the mean residence time (MRT) derived by Kubota and Ishizaki,^{29,30} who also derived an equation describing the variance of residence of a drug (VRT). They considered the

drug removal at the skin-capillary boundary by clearance, CL, and suggested a method to determine the L^2/D and CL/V values from MRT and VRT values or the apparent first-order elimination constant after the removal of ointment in vivo. In the present case, the D' and K' values were determined at first and the MTT value was calculated as an index of drug penetrability.

2.5. Summary

Nine azacycloalkanone (5, 6, or 7 member ring) derivatives with an alkyl or alkenyl (terpene) chain (10, 15, or 20 carbons) were compared with Azone for their effects on the percutaneous penetration of 6-MP through excised guinea pig skin. Pretreatment of skin with an enhancer markedly increased penetration and skin accumulation of 6-MP. Superior enhancing effects were observed for enhancers having a geranyl chain of 10 carbons and an azacyclo ring with one carbonyl group. Enhancers with a C20 tail chain were less effective. Enhancer ring size had little effect on enhancing activity, whereas the increase in the number of carbonyl groups in the ring caused a decrease.

The one-layer skin model for finite dose system well describes the diffusion process of a drug applied in the form of solution. The Laplace transforms of the equations corresponding to the time courses of the drug amounts in the receptor solution, in the skin, and in the donor solution were derived. Computer fitting of the penetration profiles of in vitro experiments to the obtained Laplace transformed equation by a nonlinear least squares program based on fast inverse Laplace transform algorithm (MULTI(FILT)) gave two parameters corresponding to drug diffusion and partitioning. These parameters well estimated the drug amounts remaining in

the donor solution and in the skin. The diffusion parameter was little affected by pretreatment with an enhancer, whereas the partition parameter was markedly increased. This suggests that enhancement is determined by the ability to increase the drug partitioning into the skin and to enlarge the drug concentration gradient in the skin barrier. Using these parameters, MTT was calculated and was shown to be a good index for drug penetrability.

The enhancers with an alkyl chain induced severer primary skin irritation (erythema and edema) than those with an alkenyl chain. From the balance between enhancing and irritating activities, it is concluded that 7GU, 7FU, and 5FU are favorable enhancers.

Chapter 3. Relationship between Physicochemical Properties of Penetrant and Efficacy of Enhancer

The activity of a percutaneous penetration enhancer is known to be changed depending on physicochemical properties of tested drugs. However, there are few reports which dealt systematically with the problem of the relationship between the physicochemical properties of penetrant and the activity of a percutaneous penetration enhancer. The optimum concentration of enhancers such as 1-dodecylazacycloheptan-2-one (Azone) and decylmethylsulfoxide is varied by the properties of penetrant and vehicle.^{5,6,50)} The optimum concentration of an enhancer in a vehicle is one of the critical problems.

In this chapter, seven drugs with a wide range of partition coefficient were chosen and the effects of Azone and 1-geranyl- (7GU) and 1-farnesylazacycloheptan-2-one (7FU) on the penetration of these drugs were examined.⁵¹⁾ Further, 7GU was picked up as a model enhancer and the effect of the amount of 7GU on its enhancing activity was investigated.⁵²⁾ Pretreatment method was employed to avoid the effect of the enhancers on the thermodynamic activity of penetrant in vehicle.^{53,54)} The obtained penetration profiles were analyzed based on the one-layer skin model described in Chapter 2. The effect of the enhancers was discussed in terms of the drug affinity to skin and drug diffusivity in skin. Further, a general consideration for the mechanism of skin permeation was developed based on a two-layer skin model.

3.1. Experimental

3.1.1. Materials

Azone was a gift from Nelson Research, USA. 7GU and 7FU were synthesized by Kuraray Co., Japan. Acyclovir (AC) and 5-fluorouracil (5-FU) were kindly supplied from Wellcome Foundation and Kyowa Hakko Kogyo, Japan, respectively. Sulfanilic acid (SA) and 6-mercaptopurine (6-MP) (Nacalai Tesque, Inc., Japan), indomethacin (IM) and triamcinolone acetonide (TMA) (Sigma Chemical Co.), and butylparaben (BP) (Tokyo Chemical Industry) were obtained commercially. All above drugs and other reagents of analytical grade were used without further purification. [^{14}C]SA (3.90 mCi/mmol) and [^{14}C]BP (1.94 mCi/mmol) were obtained from Daichi Pure Chemicals, Japan. [^3H]AC (19.8 Ci/mmol), [^{14}C]IM (22.0 mCi/mmol), and [^3H]TMA (25 Ci/mmol) were from New England Nuclear, Boston, MA. [^{14}C]5-FU (58.5 mCi/mmol) and [^{14}C]6-MP (65 mCi/mmol) were from Commissariat A L'Energie Atomique, France.

3.1.2. Determination of Partition Coefficients of Drugs

Partition coefficients of drugs between n-octanol and water at 37 °C were determined as following. SA, AC, and 5-FU were dissolved in water saturated with n-octanol, and 6-MP, IM, TMA, and BP were dissolved in n-octanol saturated with water at a concentration of 0.1 mM. Each solution contained 0.5 μCi of radiolabelled compound per 10 ml. Two milliliter aliquots of each solution were withdrawn and mixed with the same volume of the counter-phase solvent. The mixture was shaken for 10 min, centrifugated, and left at 37 °C for 48 h. After equilibration, a 1 ml aliquot of each phase was withdrawn into a vial. The effect of the percutaneous penetration enhancers on the partition coefficient between n-octanol and water was also examined using n-octanol containing 10 % of an enhancer as the organic phase. The partition coefficient was calculated as the ratio of radioactivity in the organic phase

to that in the aqueous phase.

3.1.3. In Vitro Percutaneous Penetration Experiment

Through all the experiments, the same system described in Chapter 2 was employed. The donor solutions were prepared by dissolving SA, AC, and 5-FU in water (2 mM), 6-MP and BP in water (1 mM), and 5-FU, 6-MP, IM, TMA, and BP in ethanol (2 mM). These solutions contained 4-10 μ Ci of radiolabelled compounds per 10 ml.

The effect of enhancers was examined by the pretreatment method. The skin was treated with 0.2 ml of ethanol (control), 3.3 % ethanolic solution of an enhancer, or ethanolic solution of 7GU at various concentrations. Twenty-four hours later, ethanol in the donor cell was evaporated with hair dryers, and a 1 ml aliquot of test solution was applied to each donor cell. The dermal side of the skin was continuously washed with saline containing streptomycin sulfate (50 mg/L) and penicillin G potassium (30 mg/L), which flowed at about 6 ml/h. The receptor fluid was collected at every 90 min for 24 h. Throughout the experiments, the donor cell was capped with a silicon stopper to prevent the donor solution from evaporation. For the nonpretreated control experiments, a donor solution was applied to the excised skin without any pretreatment.

At the end of the 24-h experiment, the drug remaining in the donor cell was recovered with 8 ml of water or ethanol. The skin sample was removed from the cell and a 1-cm diameter disk was punched out for the determination of the amount of drug in the skin.

All the determination of drugs was carried out by liquid scintillation counting (Model LSC-903, Aloka) as described in Chapter 2.

3.2. Theoretical

3.2.1. One-Layer Skin Model

One-layer skin model is described in Chapter 2 (Fig. 9). The penetration profiles obtained by the diffusion experiment were fitted to Eq. 8 using MULTI(FILT),²⁸⁾ and the diffusion parameter D' (Eq. 12) and the partition parameter K' (Eq. 13) were determined.

3.2.2. Two-Layer Skin Model

The model considering skin as a homogeneous plane membrane has been successfully employed in most cases. However, skin is composed of some layers with different physiological and physicochemical properties. The outermost layer, stratum corneum, is known as the most impermeable layer to most drugs, whereas viable epidermis and dermis exhibit their barrier properties only for lipophilic penetrants.³⁴⁾ There are some reports revealing that percutaneous penetration enhancers such as Azone²²⁾ and oleic acid⁵⁵⁾ reduce the barrier properties of stratum corneum but have little effect on those of viable epidermis or dermis. Therefore, it is significant to analyze diffusion process considering these layers for elucidating the mechanism of drug penetration or the action of enhancers.

Figure 18 shows the diffusion model assuming that the skin is composed of stratum corneum (volume; V_2 and thickness; L_2) and the lower layer (epidermis and dermis) (volume; V_3 and thickness; L_3). The equations of Fick's second law of diffusion for the stratum corneum (drug concentration; C_2) and the lower layer (drug concentration; C_3) are expressed as follows, respectively (x ; distance):

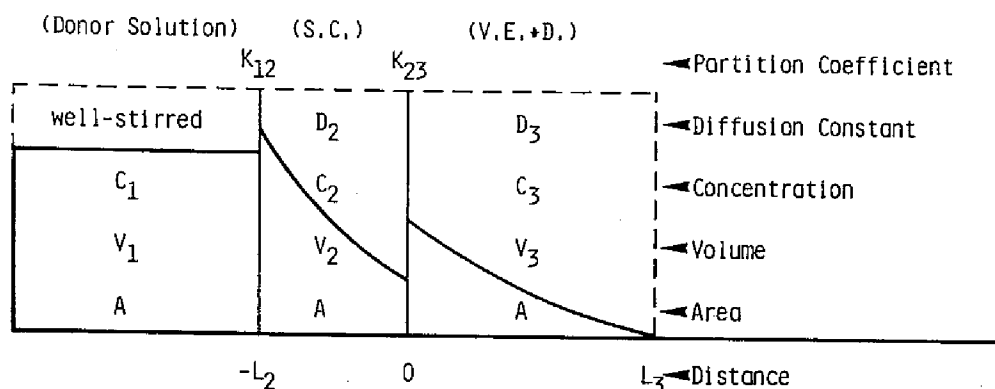


Figure 18. The two-layer skin model for finite dose system. The well-stirred condition and perfect sink condition are assumed in the donor and the receptor phases, respectively. S.C., V.E., and D. stand for stratum corneum, viable epidermis, and dermis, respectively.

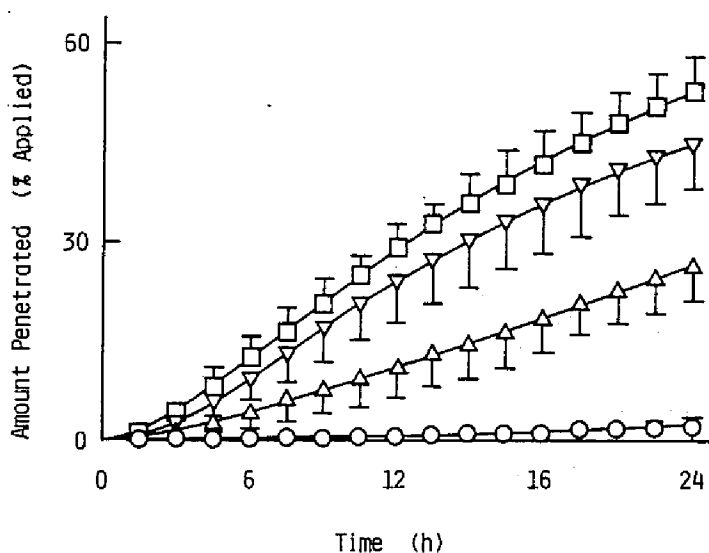


Figure 19. Percutaneous penetration of 5-FU through guinea pig skin pretreated with ethanol (O) or ethanolic solution of Azone (□), 7GU (▽), or 7FU (△). Each point represents the mean value of 3 experiments and the vertical bars indicate standard deviations. The connected lines were calculated for the parameters listed in Table VIII.

$$\frac{\partial C_2}{\partial t} = D_2 \frac{\partial^2 C_2}{\partial x^2} \quad (16)$$

$$\frac{\partial C_3}{\partial t} = D_3 \frac{\partial^2 C_3}{\partial x^2} \quad (17)$$

A penetrant partitions from donor solution to the surface of stratum corneum with a partition coefficient K_{12} and diffuses in stratum corneum with a diffusion constant D_2 . At the end of stratum corneum, the penetrant partitions to the surface of the lower layer according to its partition coefficient (K_{23}) between the lower layer and stratum corneum and diffuses in the lower layer with a diffusion constant D_3 . The boundary conditions are:

$$K_{12}C_1 = C_2 \quad (x = -L_2) \quad (18)$$

$$V_1 \frac{\partial C_1}{\partial t} = D_2 A \frac{\partial C_2}{\partial x} \quad (x = -L_2) \quad (19)$$

$$K_{23}C_2 = C_3 \quad (x = 0) \quad (20)$$

$$D_2 \frac{\partial C_2}{\partial x} = D_3 \frac{\partial C_3}{\partial x} \quad (x = 0) \quad (21)$$

$$C_3 = 0 \quad (x = L_3) \quad (22)$$

The initial conditions are (X_0 ; the applied amount of a penetrant):

$$C_1 = C_0 \text{ or } V_1 C_1 = X_0 \quad (23)$$

$$C_2 = C_3 = 0 \quad (24)$$

According to this model, the Laplace transforms of the equations for the drug amounts penetrating into the receptor medium (\bar{Q}) and remaining in the vehicle (\bar{X}) and in the skin (\bar{M}) are expressed, respectively, as (see Appendix 2):⁵⁶⁾

$$\bar{Q} = K_{12}K_{23}V_2V_3X_0/s/k(s) \quad (25)$$

$$\bar{X} = V_1X_0[V_2d_3\cosh(d_2)\sinh(d_3) + K_{23}V_3d_2\sinh(d_2)\cosh(d_3)]/s/k(s) \quad (26)$$

$$\bar{M} = \bar{M}_2 + \bar{M}_3 \quad (27)$$

$$\bar{M}_2 = K_{12}V_2X_0\{d_3/d_2V_2\sinh(d_2)\sinh(d_3) + K_{23}V_3\cosh(d_3)[\cosh(d_2)-1]\}/s/k(s) \quad (27')$$

$$\bar{M}_3 = K_{12}V_2X_0K_{23}V_3[\cosh(d_3)-1]/s/k(s) \quad (27'')$$

where \bar{M}_2 and \bar{M}_3 are the Laplace transforms for the drug amounts in stratum corneum and the lower layer, respectively, and:

$$d_2 = L_2(s/D_2)^{1/2} \quad (28)$$

$$d_3 = L_3(s/D_3)^{1/2} \quad (29)$$

$$k(s) = V_2d_3\sinh(d_3)[V_1\cosh(d_2)+K_{12}/d_2V_2\sinh(d_2)] + K_{23}V_3\cosh(d_3)[V_1d_2\sinh(d_2)+K_{12}V_2\cosh(d_2)] \quad (30)$$

Mean transit time (MTT) for an applied penetrant to diffuse from the vehicle to the receptor is given as follows (see Appendix 2):⁵⁶⁾

$$MTT = \frac{V_1/K_{12}}{D_2'V_2} + \frac{V_1/K_{12}K_{23}}{D_3'V_3} + \frac{1}{2D_2'} + \frac{V_2/K_{23}}{D_3'V_3} + \frac{1}{2D_3'} \quad (31)$$

where D_2' and D_3' are the diffusion parameters:

$$D_2' = D_2/L_2^2 \quad (32)$$

$$D_3' = D_3/L_3^2 \quad (33)$$

3.3. Results

3.3.1. Partition Coefficients of Drugs between n-Octanol and Water

Table V lists the partition coefficients (PC_{oct}) of the seven drugs between n-octanol and water, which range about five orders of magnitude (from 0.00685 for SA to 371 for BP). The addition of Azone, 7GU, or 7FU into n-octanol at a concentration of 10 % increased the partition coefficient of a drug. This effect was more remarkable for the drug with high hydrophobicity, except for the partitioning of SA into n-octanol with 10 % of Azone.

3.3.2. Effect of Ethanol and Enhancers on Percutaneous Drug Penetration

In the diffusion experiments, permeation profiles of the tested drugs were obtained as representatively shown in Fig. 19. Tables VI and VII summarize the amounts of drug recovered from the donor phase, the skin, and the receptor phase at the end of the 24-h diffusion experiments for all drugs.

When water was employed as a vehicle (aqueous system, Table VI), BP, the most lipophilic compound in this study, penetrated through the nonpretreated skin (denoted by "C(NP)" in Tables) much more easily than the other penetrants. It seems that the increase in the lipophilicity of penetrant leads to the increase in the penetrability. The amount of drug in the skin increased according to the increase in PC_{oct} except for SA. On the other hand, the penetration through nonpretreated skin from ethanol vehicle (ethanolic system, Table VII) was decreased according to the increase in PC_{oct} of the penetrant. The accumulation of drug in the skin was almost the same for 5-FU, 6-MP, TMA, and BP in the ethanolic system.

Ethanol pretreatment increased the penetration of SA (7 folds), AC (2 folds), and 6-MP (4 folds) from water vehicle when compared with the nonpretreated control, whereas the penetration of BP was halved. The effect of ethanol pretreatment on the drug accumulation in the skin was not so remarkable. In the ethanolic system, the penetration of drugs except for TMA

Table V. Partition Coefficients of Drugs between n-Octanol (+ Enhancer) and Water at 37 °C

Drug	Molecular Weight	Enhancer in Organic Phase (n-Octanol) ^a			
		None	Azone	7GU	7FU
Sulfanilic Acid (SA)	173	0.00685	0.0684	0.00738	0.00923
Acyclovir (AC)	225	0.0304	0.0292	0.0350	0.0256
5-Fluorouracil (5-FU)	130	0.144	0.170	0.188	0.171
6-Mercaptopurine (6-MP)	152	0.654	0.836	1.14	0.896
Indomethacin (IM)	358	40.7	ND ^b	ND ^b	ND ^b
Triamcinolone Acetonide (TMA)	436	120	194	245	185
Butylparaben (BP)	194	371	568	2163	1658

^a Ten percent (v/v) of enhancer was added to n-octanol.

^b Not determined.

Table VI. Recovery Percentages of Drugs after 24-Hour Diffusion Experiments with Guinea Pig Skin (Aqueous System)^a

Drug	En- hancer	N	Drug Recovery (%)			
			Total	Donor	Skin	Receptor
SA	C(NP)	6	102.68 ± 2.43	89.59 ± 3.92	12.20 ± 1.67	1.02 ± 0.52
	C(P)	4	101.45 ± 3.95	68.30 ± 7.82	25.53 ± 4.00	7.62 ± 1.23
	Azone	3	106.34 ± 3.95	63.26 ± 4.37	24.00 ± 5.45	19.08 ± 4.31
	7GU	3	104.19 ± 8.05	62.76 ± 4.16	27.48 ± 10.26	13.94 ± 2.98
	7FU	4	106.59 ± 9.67	61.22 ± 2.83	28.03 ± 9.55	17.34 ± 5.94
AC	C(NP)	4	99.43 ± 3.69	96.64 ± 3.54	2.16 ± 0.17	0.63 ± 0.20
	C(P)	3	100.62 ± 0.67	96.17 ± 0.83	3.27 ± 0.14	1.19 ± 0.31
	Azone	5	104.58 ± 3.65	83.77 ± 4.83	11.64 ± 3.20	9.18 ± 4.88
	7GU	5	102.76 ± 3.29	85.58 ± 1.88	9.04 ± 2.56	8.15 ± 2.39
	7FU	4	103.50 ± 4.04	86.51 ± 7.54	8.78 ± 1.01	8.21 ± 3.34
5-FU	C(NP)	4	101.35 ± 0.34	95.91 ± 1.45	3.20 ± 1.01	2.24 ± 0.96
	C(P)	3	102.84 ± 1.68	97.66 ± 2.71	3.09 ± 1.01	2.09 ± 1.50
	Azone	3	94.73 ± 5.90	33.71 ± 6.64	7.96 ± 0.91	53.06 ± 5.27
	7GU	3	100.25 ± 1.24	45.72 ± 4.94	9.55 ± 1.86	44.98 ± 4.78
	7FU	3	101.77 ± 7.32	65.09 ± 11.01	10.34 ± 1.66	26.33 ± 5.14
6-MP	C(NP)	5	93.73 ± 5.41	87.68 ± 5.48	5.23 ± 0.39	0.83 ± 0.57
	C(P)	4	100.93 ± 2.91	90.90 ± 3.22	6.99 ± 1.79	3.04 ± 1.34
	Azone	4	96.68 ± 2.82	33.43 ± 2.65	19.36 ± 9.95	43.89 ± 12.55
	7GU	4	105.76 ± 2.22	34.82 ± 3.92	23.67 ± 9.04	47.27 ± 5.31
	7FU	3	103.79 ± 3.29	56.43 ± 4.01	25.05 ± 5.85	22.30 ± 3.44
BP	C(NP)	4	85.67 ± 9.07	29.28 ± 4.64	42.61 ± 12.54	19.04 ± 5.91
	C(P)	3	45.99 ± 1.12	8.10 ± 1.33	29.90 ± 3.55	7.99 ± 1.72
	Azone	3	94.55 ± 17.48	12.20 ± 2.85	80.99 ± 18.06	1.37 ± 0.48
	7GU	3	88.83 ± 13.78	12.23 ± 1.72	75.39 ± 12.17	1.21 ± 0.06
	7FU	3	81.75 ± 5.10	14.67 ± 4.19	66.16 ± 7.98	0.93 ± 0.13

^a C(NP) and C(P) stand for the nonpretreated and ethanol pretreated controls, respectively. Data are expressed by means ± standard deviations.

Table VII. Recovery Percentages of Drugs after 24-Hour Diffusion Experiments with Guinea Pig Skin (Ethanolic System)^a

Drug	En- hancer	N	Drug Recovery (%)			
			Total	Donor	Skin	Receptor
5-FU	C(NP)	3	106.01 ± 3.03	98.74 ± 3.90	5.45 ± 0.52	2.37 ± 0.47
	C(P)	4	98.68 ± 5.79	91.36 ± 6.12	5.30 ± 1.11	2.02 ± 0.34
	7GU	3	119.59 ± 4.75	102.45 ± 6.01	10.73 ± 3.79	6.41 ± 2.80
	7FU	4	113.72 ± 3.22	100.94 ± 1.59	7.23 ± 1.10	5.56 ± 1.47
6-MP	C(NP)	5	99.27 ± 11.53	88.51 ± 10.79	8.63 ± 1.05	2.13 ± 0.65
	C(P)	4	93.06 ± 3.21	85.29 ± 1.82	7.02 ± 2.58	0.74 ± 0.13
	7GU	3	100.25 ± 5.62	87.19 ± 7.50	10.46 ± 1.63	2.60 ± 0.34
	7FU	3	100.27 ± 4.36	88.09 ± 5.37	10.20 ± 1.00	2.43 ± 0.33
IM	C(NP)	2	66.60 ± 10.23	12.94 ± 0.78	52.34 ± 10.15	0.49 ± 0.34
	C(P)	4	77.36 ± 7.47	72.69 ± 8.04	4.43 ± 0.63	0.24 ± 0.09
	7GU	4	71.64 ± 4.58	60.26 ± 5.19	10.59 ± 2.53	0.78 ± 0.30
	7FU	4	76.79 ± 2.46	69.87 ± 0.31	6.31 ± 2.01	0.61 ± 0.18
TMA	C(NP)	3	101.54 ± 16.05	94.32 ± 14.60	6.91 ± 3.03	0.18 ± 0.11
	C(P)	4	91.98 ± 4.67	87.56 ± 4.18	4.10 ± 0.79	0.33 ± 0.13
	7GU	4	96.08 ± 8.05	92.68 ± 7.77	3.02 ± 0.41	0.38 ± 0.09
	7FU	4	95.05 ± 4.41	92.22 ± 4.04	2.38 ± 0.94	0.44 ± 0.13
BP	C(NP)	4	79.81 ± 14.03	73.82 ± 16.50	5.52 ± 2.53	0.38 ± 0.28
	C(P)	4	79.92 ± 1.16	77.45 ± 0.71	2.16 ± 0.48	0.32 ± 0.14
	7GU	3	78.12 ± 1.44	75.75 ± 1.52	2.16 ± 0.09	0.20 ± 0.03
	7FU	5	80.83 ± 4.84	77.73 ± 5.79	2.85 ± 1.21	0.25 ± 0.06

^a C(NP) and C(P) stand for the nonpretreated and ethanol pretreated controls, respectively. Data are expressed by means ± standard deviations.

was not affected or somewhat decreased by the pretreatment with ethanol. The amount of drug accumulating in the skin was also not affected (5-FU and 6-MP) or decreased (IM, TMA, and BP) by the pretreatment with ethanol.

The pretreatment of the skin with the enhancers increased the penetration of SA, AC, 5-FU, and 6-MP from water vehicle, whereas that of BP was greatly decreased. 5-FU and 6-MP showed the highest penetration in the skin pretreated with the enhancers. The pretreatment with the enhancer also increased the drug accumulation in the skin. In the ethanolic system, the pretreatment with 7GU or 7FU increased the penetration and skin accumulation of 5-FU, 6-MP, and IM when compared with the ethanol pretreated control, however, the degree of the enhancement was far less than that observed in the aqueous system. The penetration of TMA and BP was decreased in this case. As the case of the aqueous system, 5-FU and 6-MP penetrated faster than the other drugs. The increase in PC_{oct} led to less drug accumulation in the skin in the ethanolic system.

3.3.3. Relationship between Physicochemical Properties of Drugs and Their Diffusivity and Affinity to Skin Estimated Based on One-Layer Skin Model

From penetration profiles of drugs like that representatively shown in Fig. 19, parameters relating to drug diffusion (D') and partition (K') were calculated based on the one-layer skin model, and results are listed in Table VIII and IX.

When the skin was pretreated with ethanol, a clear relationship between K' and PC_{oct} was recognized in the tested drugs. The K' value increased according to the increase in the PC_{oct} except for SA when water was employed as a vehicle. However, in the case of the ethanolic system, the increase in PC_{oct} of penetrant led to the decrease in K' .

Table VIII. Diffusion Parameter (D'), Partition Parameter (K'), and Mean Transit Time (MTT) for Percutaneous Penetration of Drugs (Aqueous System)^a

Drug	Enhancer	$D' \times 10^3 \text{ (h}^{-1}\text{)}$	$K' \times 10^3 \text{ (cm)}$	MTT (h)
SA	C(NP)	11.00	74.07	436.1
	C(P)	30.12	45.52	248.8
	Azone	38.62	89.56	105.0
	7GU	17.50	202.1	118.6
	7FU	35.20	90.01	114.7
AC	C(NP)	14.50	10.60	2105
	C(P)	20.46	11.43	1386
	Azone	19.93	98.76	186.8
	7GU	27.13	56.42	226.4
	7FU	22.65	72.97	214.7
5-FU	C(NP)	18.21	26.07	697.9
	C(P)	25.13	14.93	868.3
	Azone	66.40	224.4	28.89
	7GU	63.18	168.3	37.85
	7FU	43.80	117.4	73.32
6-MP	C(NP)	12.00	21.00	1305
	C(P)	35.17	13.52	683.6
	Azone	26.96	631.3	37.25
	7GU	22.06	1008	36.98
	7FU	15.39	491.3	74.59
BP	C(NP)	13.84	163.5	176.8
	C(P)	17.24	299.6	90.63
	Azone	27.08	9.265	1287
	7GU	23.04	10.40	1350
	7FU	30.40	6.170	1713

^a C(NP) and C(P) stand for the nonpretreated and ethanol pretreated controls, respectively. Parameters were obtained by the computer fitting of the penetration profile to Eq. 8. The total recovery percentage in Table VI was employed as the dose (X_0) in Eq. 8.

Table IX. Diffusion Parameter (D'), Partition Parameter (K'), and Mean Transit Time (MTT) for Percutaneous Penetration of Drugs (Ethanollic System)^a

Drug	Enhancer	$D' \times 10^3 \text{ (h}^{-1}\text{)}$	$K' \times 10^3 \text{ (cm)}$	MTT (h)
5-FU	C(NP)	22.76	22.07	655.6
	C(P)	20.28	20.45	792.1
	7GU	17.82	68.23	289.8
	7FU	32.08	25.99	397.4
6-MP	C(NP)	10.89	65.11	494.8
	C(P)	14.12	13.87	1661
	7GU	19.58	26.99	627.8
	7FU	23.35	19.10	735.1
IM	C(NP)	6.003	44.29	1280
	C(P)	9.574	11.56	2928
	7GU	12.03	24.95	1102
	7FU	21.93	6.499	2256
TMA	C(NP)	8.042	28.76	1438
	C(P)	17.71	3.967	4559
	7GU	21.68	3.281	4498
	7FU	25.10	3.183	4004
BP	C(NP)	14.32	4.464	5014
	C(P)	17.21	4.865	3831
	7GU	47.72	0.656	10178
	7FU	26.40	1.909	6335

^a C(NP) and C(P) stand for the nonpretreated and ethanol pretreated controls, respectively. Parameters were obtained by the computer fitting of the penetration profile to Eq. 8. The total recovery percentage in Table VII was employed as the dose (X_0) in Eq. 8.

Ethanol pretreatment little affected or decreased the K' value, except for BP in the aqueous system, when compared with the nonpretreated control. The K' values of 6-MP, IM, and TMA in the ethanolic system were decreased remarkably. On the other hand, the D' values were increased by the pretreatment with ethanol.

The effect of the pretreatment with the enhancers on these parameters is rather interesting. In Figs. 20A-C, the increasing ratios of the penetrating amount, K' , and D' to each of ethanol pretreated control values are plotted as a function of PC_{oct} of penetrant (aqueous system). The enhancers enlarged K' value, and this effect was encouraged according to the increase in PC_{oct} of penetrant and was remarkable for 6-MP (Fig. 20B). The increase in the drug amount in the skin is thought to be caused by the increase in the affinity of drug to the skin, i. e. K' in the present analysis, which can be conformed in Table VIII for SA, AC, 5-FU, and 6-MP. For BP, however, K' was diminished to only 1/30 of that of the ethanol pretreated control. The effect of enhancer on D' was less remarkable than that on K' , and the diffusivity of drug was nearly the same as that obtained in the ethanol pretreated control experiment. In the case of 5-FU, D' was enlarged by about 2.5 times that of the ethanol pretreated control, which led to the large activity of the enhancers on 5-FU penetration. The effect of the enhancers on the drug penetration from water vehicle seems to be determined by the ability to increase the affinity of drug to the skin.

When the drug was applied as an ethanolic solution, the effect of the enhancers was far less than when applied as an aqueous solution (Fig. 21A). In this case, the increase in the K' value was rather small (Fig. 21B). The effect of enhancers was lowered according to the increase in PC_{oct} , because the affinity of drug to the skin was diminished. The variation in the K'

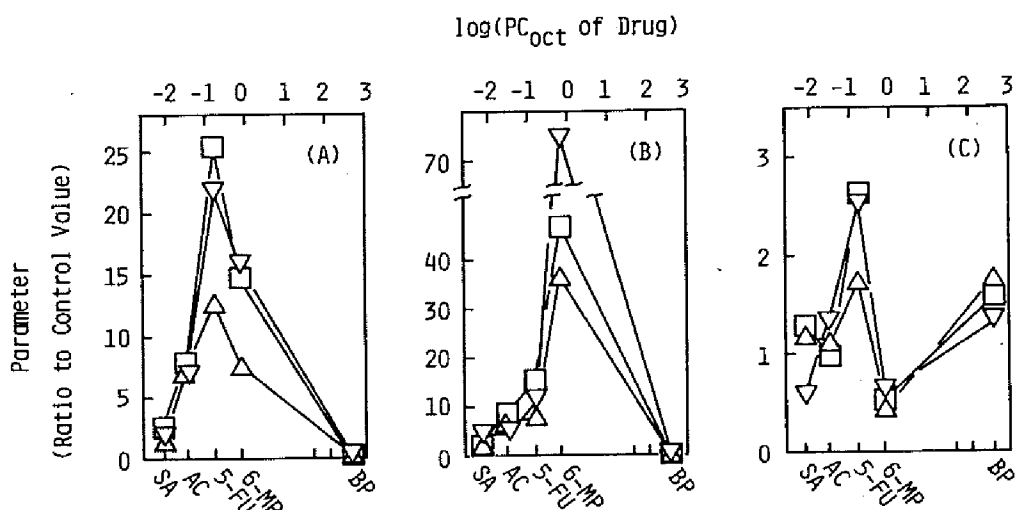


Figure 20. The relationships between the partition coefficient of drug between n-octanol and water and the amount of drug penetrating for 24 h (A), the partition parameter (B), and the diffusion parameter (C) in the aqueous system. Results are expressed as the ratios to each of ethanol pretreated control values. Key: (\square) Azone, (∇) 7GU, and (\triangle) 7FU.

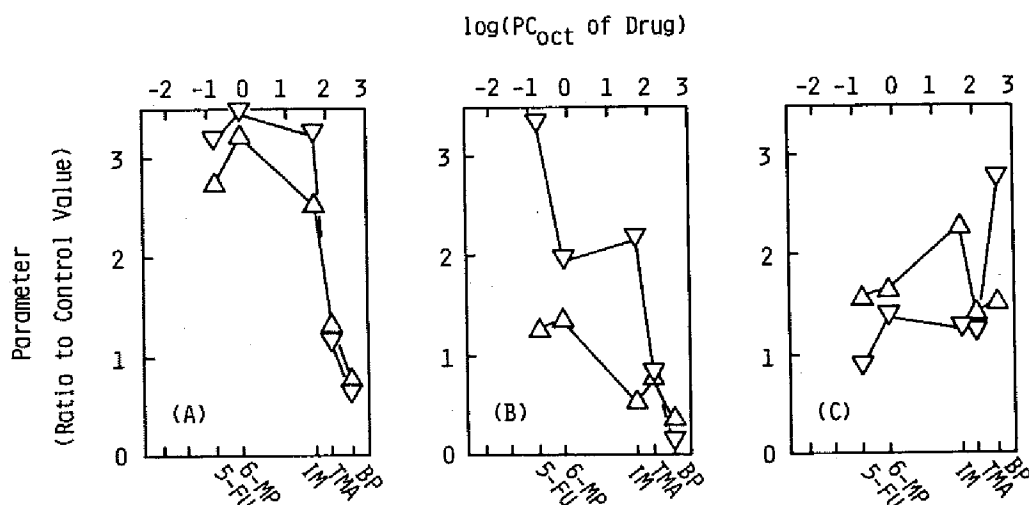


Figure 21. The relationships between the partition coefficient of drug between n-octanol and water and the amount of drug penetrating for 24 h (A), the partition parameter (B), and the diffusion parameter (C) in the ethanolic system. Results are expressed as the ratios to each of ethanol pretreated control values. Key: (∇) 7GU and (\triangle) 7FU.

value is the main factor of the different sensitivity of drugs to the activity of enhancers in the ethanolic system. The increase in the D' value was less than 3 times of the ethanol pretreated control value (Fig. 21C) as observed in the aqueous system, however, the contribution of increase in drug diffusivity to the enhancement of penetration was relatively high because of the small increase in the drug affinity to the skin.

3.3.4. Effect of Preloading Amount of 7GU on Percutaneous Penetration of Drugs

The effect of preloading amount of 7GU on its enhancing activity was investigated using AC, 5-FU, 6-MP, and BP, which were applied to the skin as aqueous solutions. The penetration profiles of 6-MP from water through the guinea pig skin pretreated with various amounts of 7GU are shown in Fig. 22. The connected lines were obtained by the computer fitting of the data to Eq. 8, based on the one-layer skin model. Figures 23-26 illustrate the relationships between the preloaded amount of 7GU and the drug penetration, drug accumulation in the skin, and D' and K' obtained by computer fitting of the penetration profiles to Eq. 8, based on the one-layer skin model.

The amounts of AC penetrating and accumulating in the skin were increased by the pretreatment with 7GU, the extent of which correlated almost linearly with the preloading amount of 7GU (Fig. 23). The D' value was not so much affected, and the variation was only within 30 % of the control value. Whereas the K' value increased with the amount of 7GU, the pattern of which was very similar to that of the penetrating amount, suggesting that 7GU enlarged the partition coefficient of AC between skin and vehicle and increased both the drug penetration and accumulation in the skin.

The penetration of 5-FU was also raised almost linearly with the amount of 7GU, and the contribution of the increased affinity to this was obvious

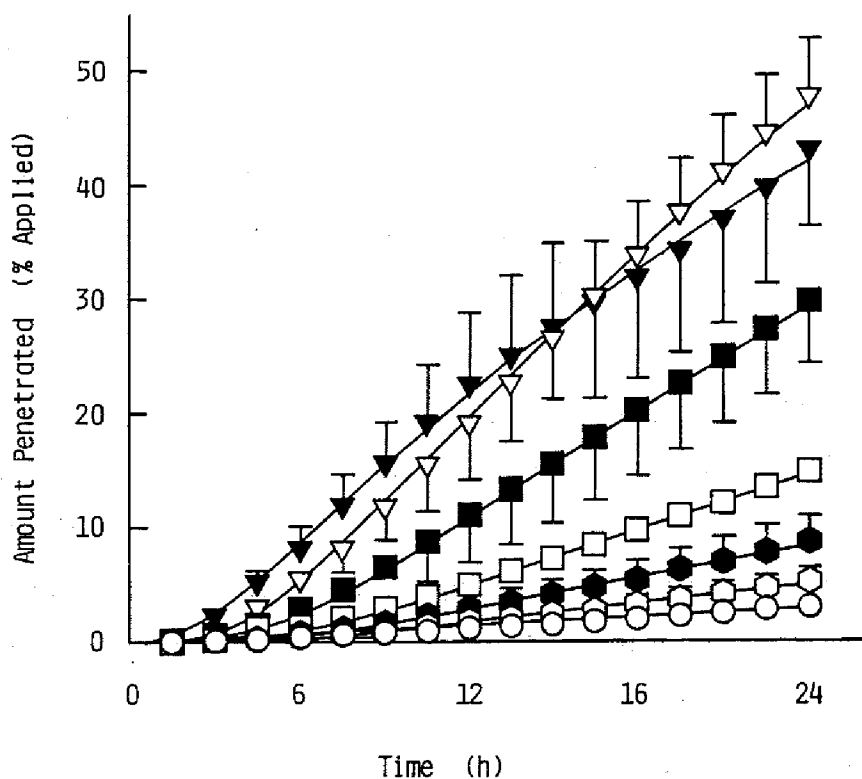


Figure 22. Percutaneous penetration of 6-MP through guinea pig skin pretreated with ethanol (○) or 3.2 (◻), 6.4 (◼), 12.7 (◻), 20.0 (◼), 25.5 (▽), or 51.0 (▼) $\mu\text{mol}/3.14 \text{ cm}^2$ of 7GU. Each point represents the mean value of 2-4 experiments and the vertical bars indicate standard deviations. The connected lines were obtained by computer fitting of data to Eq. 8.

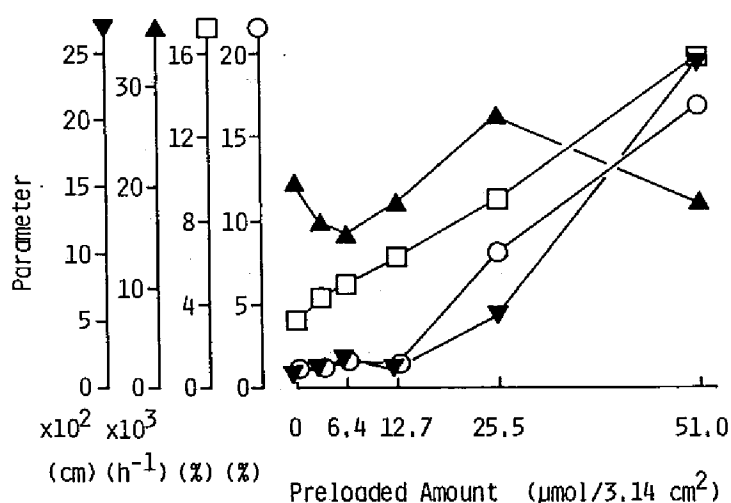


Figure 23. The relationships between the pretreated amount of 7GU and penetrating amount for 24 h (○), accumulating amount in the skin for 24 h (□), the diffusion parameter (▲), and the partition parameter (▼) of AC.

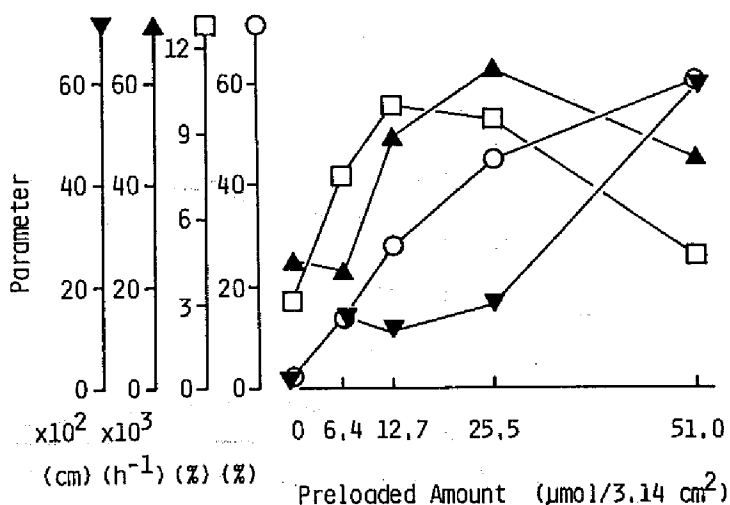


Figure 24. The relationships between the pretreated amount of 7GU and penetrating amount for 24 h (○), accumulating amount in the skin for 24 h (□), the diffusion parameter (▲), and the partition parameter (▼) of 5-FU.

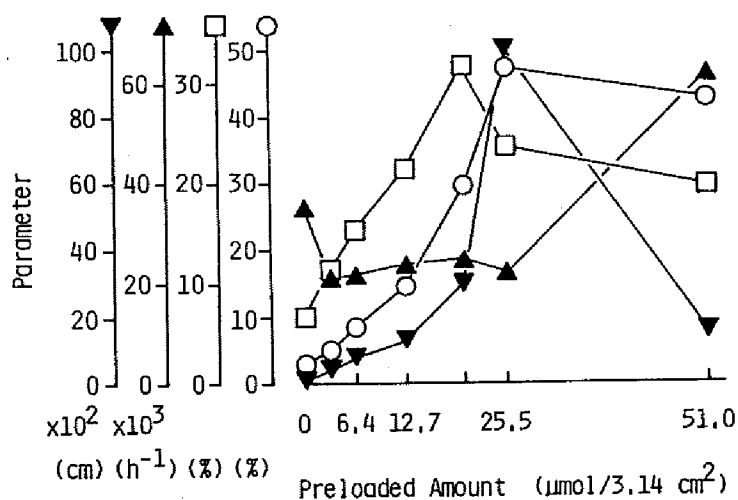


Figure 25. The relationships between the pretreated amount of 7GU and penetrating amount for 24 h (○), accumulating amount in the skin for 24 h (□), the diffusion parameter (▲), and the partition parameter (▼) of 6-MP.

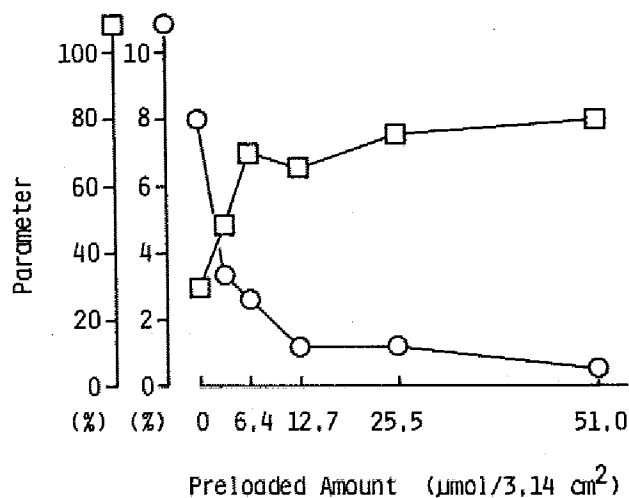


Figure 26. The relationships between the pretreated amount of 7GU and amounts of BP penetrating for 24 h (○) and accumulating in the skin for 24 h (□).

(Fig. 24). Although the diffusivity of AC or 6-MP was not so much affected by 7GU, that of 5-FU was rather improved. It seems that the enhancer reduced the interaction between 5-FU and skin component. The amount of 5-FU in the skin was maximized at $12.7 \mu\text{mol}/3.14 \text{ cm}^2$ of 7GU. The reduction in the skin content of 5-FU by larger amount of 7GU is considered to be caused by the decrease in the drug concentration in the donor solution. Only 46 % and 25 % of the applied dose were remaining in the donor solution at 24 h when the skin was pretreated with 25.5 and $51.0 \mu\text{mol}/3.14 \text{ cm}^2$ of 7GU, respectively. Donor concentration decrease with time canceled the effect of the rising affinity (K') of 5-FU on the skin accumulation.

The penetrating amount and K' of 6-MP were affected by 7GU, which were maximized at $25.5 \mu\text{mol}/3.14 \text{ cm}^2$ of it (Fig. 25). The skin accumulation of 6-MP for 24 h was very large compared with those of AC and 5-FU, and was maximized at $20 \mu\text{mol}/3.14 \text{ cm}^2$ of 7GU. The D' value was not so affected by 7GU except for the case with the largest amount of 7GU.

The case of BP was quite different from those mentioned above (Fig. 26). Increasing the amount of 7GU encouraged the accumulation of BP in the skin, while it discouraged the penetration. These patterns were hardly expected by the one-layer skin model.

3.3.5. Reliability of Analysis Based on One-Layer Skin Model

The drug amounts in the donor solution and the skin can be calculated from D' and K' values by Eqs. 9 and 10, respectively. The correlation between these estimated values and the observed values is one of the indices for the reliability of the analysis based on the one-layer skin model. Such plots for the amount of drug in the skin at 24 h are shown in Figs. 27A-E for the aqueous system with Azone, 7GU, and 7FU, and Figs. 28A-E for the ethanolic

system with 7GU and 7FU. The analysis based on the one-layer skin model gives good estimation and is proved to be suit for the drugs with PC_{oct} less than unity such as AC, 5-FU, and 6-MP. For the drugs with PC_{oct} over unity, the estimated value was smaller than the observed one. Especially, the discrepancy was large for BP, the most hydrophobic drug in this study. For IM and TMA, the observed amount in the skin was larger than the estimated amount, suggesting higher affinity of drugs to the stratum corneum than estimated. However, the discrepancy was not so extreme as seen in BP in the aqueous system, and the analysis seems to be still valuable. Also Figs. 29A-H show the correlation between observed and estimated amounts of drugs in the donor (Figs. 29A-D) and the skin (Figs. 29E-H) for the experiments using the skin preloaded with various amounts of 7GU. For AC, 5-FU, and 6-MP (Figs. 29A-C and E-G), the correlations are rather good, supporting the reliability of the analysis. While the correlations for BP are poor (Figs. 29D and H), and the estimation gives larger amount of BP in the donor and smaller amount in the skin than observed amounts.

3.3.6. Simulation Based on Two-Layer Skin Model

There are some reports revealing that percutaneous penetration enhancers such as Azone and oleic acid reduce the barrier properties of stratum corneum but have little effect on those of viable epidermis and dermis.^{22,55)} The mechanism of action of 7GU seems to be not so different from that of Azone. Further, the analysis of the penetration of AC, 5-FU, and 6-MP through the skin preloaded with 7GU based on the one-layer skin model suggests that 7GU dominantly affects the drug affinity to the skin. So it is assumed that the partition coefficient between stratum corneum and vehicle (water) (K_{12}) is only the parameter which is affected by the enhancer, and the effect of

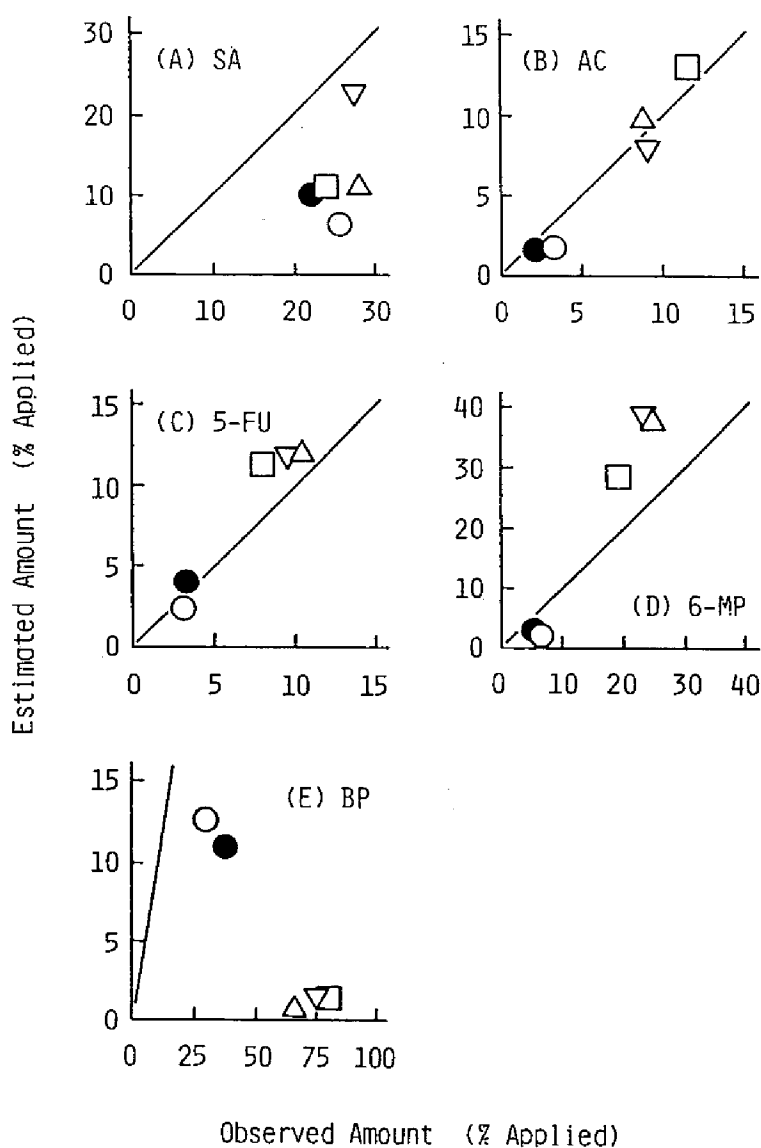


Figure 27. The relationships between the estimated and observed amounts of drugs in the skin in the aqueous system. Estimated values were calculated for the parameters listed in Table VIII using Eq. 10. The total recovery percentage in Table VI was employed as the dose (X_0). The skin was not pretreated (●) or pretreated with ethanol (○), ethanolic solution of Azone (□), 7GU (▽), or 7FU (△).

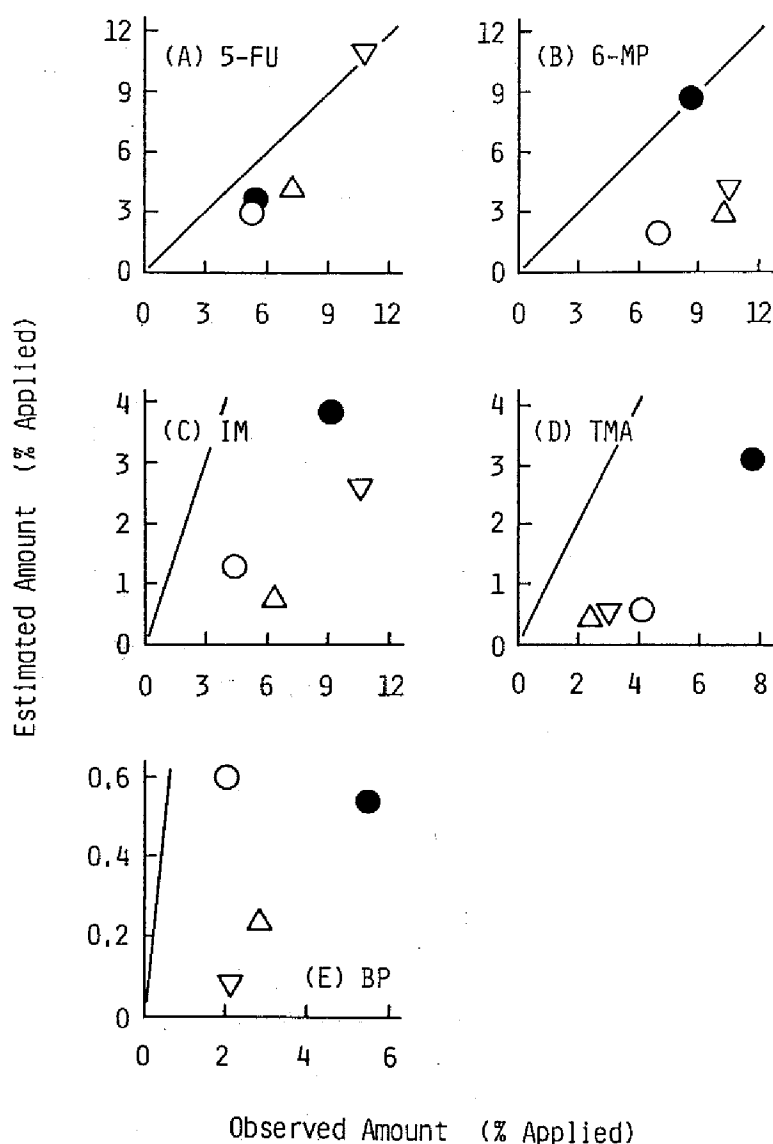


Figure 28. The relationships between the estimated and observed amounts of drugs in the skin in the ethanolic system. Estimated values were calculated for the parameters listed in Table IX using eq 10. The total recovery percentage in Table VII was employed as the dose (X_0). The skin was not pretreated (●) or pretreated with ethanol (○), ethanolic solution of 7GU (▽) or 7FU (△).

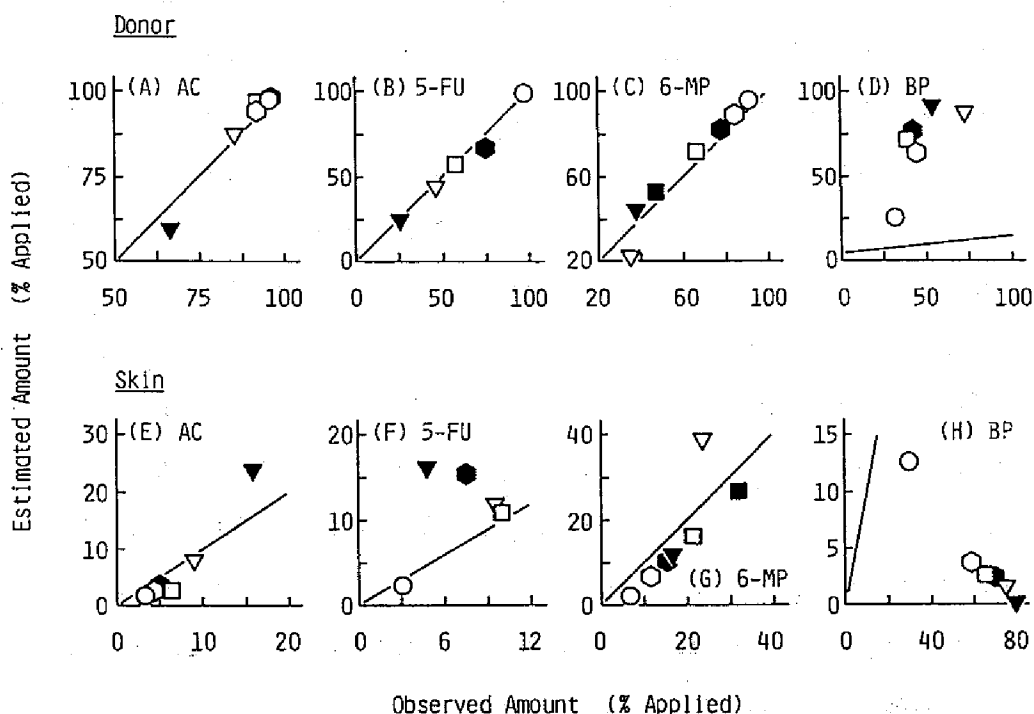


Figure 29. The relationships between the estimated and observed amounts of drugs in the donor solution (A-D) or in the skin (E-H) in the diffusion experiment with the skin pretreated with various amount of 7GU. The pretreated amounts of 7GU were 0 (○), 3.2 (◐), 6.4 (◑), 12.7 (◒), 20.0 (◓), 25.5 (▽), and 51.0 (▼) $\mu\text{mol}/3.14 \text{ cm}^2$.

enhancer on the percutaneous penetration was simulated based on the two-layer skin model. The two-layer skin model (Fig. 18) requires the parameters, K_{12} , K_{23} , D_2 , D_3 , A , V_1 , L_2 ($V_2 = L_2A$), L_3 ($V_3 = L_3A$), and X_0 . These parameters were determined as follows. The parameters A , V_1 , and X_0 are 3.14 cm^2 , 1 ml , and 100% , respectively, in the present experimental system. It was reported from the analysis of the penetration profiles of 6-MP through intact and stripped guinea pig skin that D_2 was $0.390 \times 10^{-6} \text{ cm}^2/\text{h}$, D_3 was $255 \times 10^{-6} \text{ cm}^2/\text{h}$, L_2 was 0.00242 cm , and L_3 was 0.0800 cm .⁵⁶⁾ The present simulation refers to these values for the drug penetration and sets that D_2 is $0.40 \times 10^{-6} \text{ cm}^2/\text{h}$, D_3 is $280 \times 10^{-6} \text{ cm}^2/\text{h}$, L_2 is 0.0024 cm , and L_3 is 0.080 cm . From these values D_2' and D_3' are 0.07 and 0.04 h^{-1} , respectively. The variable is only K_{12} . It is assumed that the nature of viable epidermis and dermis is very close to that of water, and the partition coefficient between stratum corneum and the lower layer (K_{23}) is substituted with the reciprocal of K_{12} .

Figure 30 illustrates the simulated relationship between K_{12} and drug amounts in the receptor medium, the donor, the stratum corneum, and the lower layer 24 h after the application, which are calculated for the parameter values listed above by Eqs. 25, 26, 27', and 27'', respectively. It is clear that the penetrating amount of drug increases, passes through a maximum, and decreases with an increase in K_{12} value. The drug amount in the lower layer varies in the same way. On the other hand, the drug amount in the stratum corneum does monotonously increase with K_{12} value. Providing that the penetrant is relatively hydrophilic and K_{12} is small, the increase in its affinity to the stratum corneum should result in the increase in drug amounts both penetrating and accumulating in the skin. Providing that the penetrant is relatively hydrophobic and K_{12} is large, the increase in the drug affinity to the stratum corneum should raise the drug accumulation in the skin, while

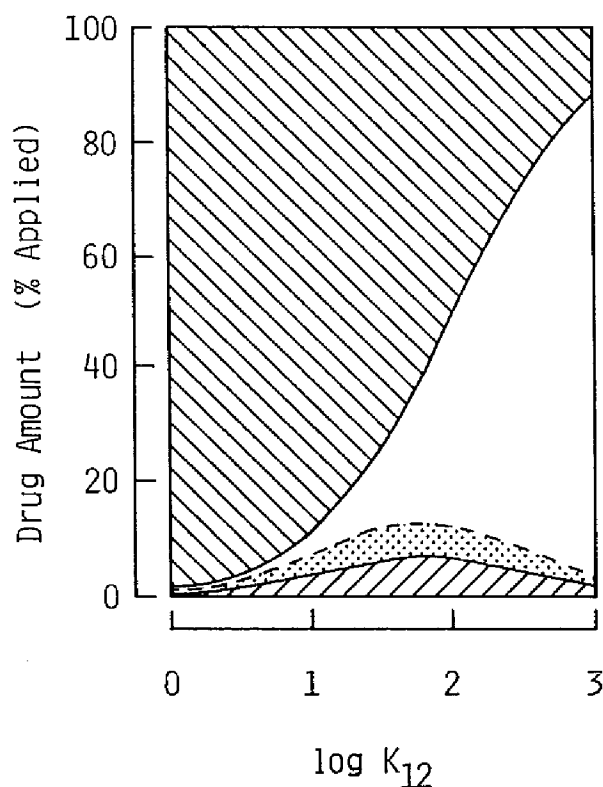






Figure 30. The estimated relationship between the partition coefficient of drug (stratum corneum / vehicle (water)) and amounts of drug in the donor, the stratum corneum, the lower layer, and the receptor at 24 h after beginning the diffusion experiment. Drug amounts were calculated by Eqs. 25, 26, 27', and 27". Parameters were set as $V_1=1 \text{ cm}^3$, $V_2=0.0076 \text{ cm}^3$, $V_3=0.25 \text{ cm}^3$, $D_2=0.40 \times 10^{-6} \text{ cm}^2/\text{h}$, $D_3=280 \times 10^{-6} \text{ cm}^2/\text{h}$, $L_2=0.0024 \text{ cm}$, $L_3=0.080 \text{ cm}$, and $X_0=100 \%$. The variable is K_{12} , and K_{23} is assumed to be the reciprocal of K_{12} . Key:  drug amount in the donor,  drug amount in the stratum corneum,  drug amount in the lower layer, and  drug amount in the receptor.

the penetrating amount should be reduced.

In Figure 31, MTT calculated by Eq. 31 are plotted as a function of K_{12} value. The minimum value of MTT is obtained at K_{12} value which maximizes the penetrating amount of drug (Fig. 30). If one replaces K_{23} in Eq. 32 with $1/K_{12}$ and differentiates it with respect to K_{12} , then:

$$\frac{dMTT}{dK_{12}} = - \frac{V_1}{D_2' V_2 K_{12}^2} + \frac{V_2}{D_3' V_3} \quad (34)$$

Putting Eq. 34 to zero gives the K_{12} value which minimizes MTT ($K_{12,min}$):

$$K_{12,min} = \left(\frac{D_3 L_1}{D_2 L_3} \right)^{1/2} \quad (35)$$

The value of $K_{12,min}$ in this simulation is calculated to be 52.3 using Eq. 35 and the minimum value of MTT is 195 h.

It seems that much higher affinity of a drug to the stratum corneum detains the drug in this layer and impedes the drug from moving into the lower layer. In Fig. 31, t_1 corresponds to the mean time for drug to traverse the boundary between donor solution and stratum corneum and t_3 corresponds to that between stratum corneum and the lower layer (see Appendix 2).⁵⁶⁾ When K_{12} is small, MTT is large because of large t_1 value. This means that the stratum corneum acts as an effective barrier and both of drug penetration and accumulation in the skin are small. Increasing the affinity of drug to the stratum corneum monotonously diminishes t_1 value, while t_3 value becomes larger in a monotonous fashion. When K_{12} becomes sufficiently large, t_3 exceeds t_1 and drug accumulates in the stratum corneum and hardly moves into the next layer. The concept of MTT is useful for us to grasp the drug penetration process.

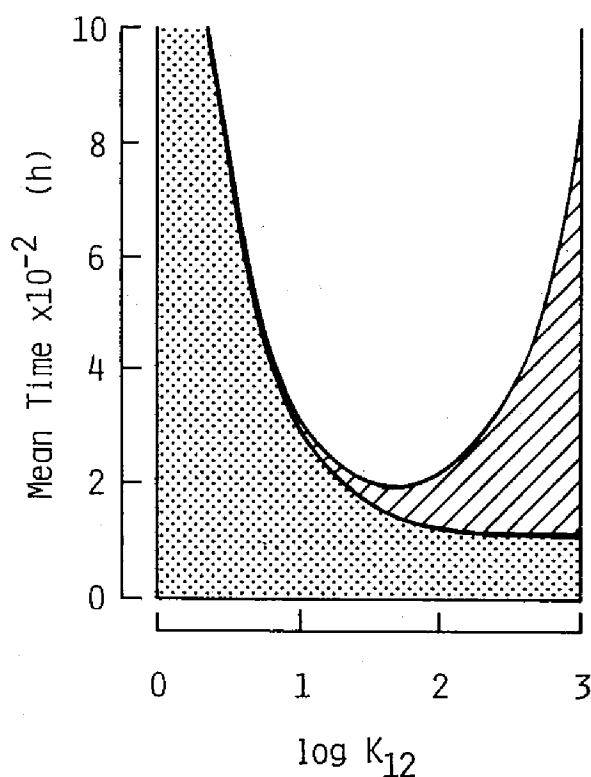


Figure 31. The estimated relationship between the partition coefficient of drug (stratum corneum / vehicle (water)) and the mean transit time calculated by Eq. 31. Key: dotted t_1 ($= V_1/D_2'V_2K_{12} + V_1/D_3V_3K_{12}K_{23}$) and hatched t_3 ($= V_2/D_3V_3K_{23}$). t_1 and t_3 correspond to the mean times for drug to traverse the boundaries between vehicle and stratum corneum and between stratum corneum and the lower layer, respectively (see Appendix 2). The mean times to diffuse through stratum corneum, t_2 ($= 1/2D_2'$), and to diffuse through the lower layer, t_4 ($= 1/2D_3'$), can not be concisely indicated in this figure because of their relatively small values.

3.4. Discussion

The active transport system or the solvent drag transport are not known in the percutaneous drug penetration, which can be basically considered as the passive diffusion process. In this point of view, the driving force behind the drug movement is the difference in the concentration or thermodynamic activity between the vehicle and the deeper tissues,⁵⁷⁾ and the partition coefficient of drug between skin and vehicle and the diffusion constant in the skin are the important factors to determine the drug penetrability.

The balance of the lipophilicities or solubility parameters of drug, vehicle, and skin determines the partition coefficient of the drug between the skin and vehicle. Although the partition coefficient between n-octanol and water of the seven drugs ranges about five orders of magnitude, that between the nonpretreated or ethanol pretreated skin and water, which was calculated based on the one-layer skin model, ranges less than two orders of magnitude. Especially the drugs with PC_{oct} lower than unity, AC, 5-FU, and 6-MP, showed almost the same affinity to the each of nonpretreated and ethanol pretreated skins. One of the possible explanations for this can be made by considering two parallel domains in the skin (stratum corneum), one of which is aqueous and another is lipoidal.^{58,59)} The partition coefficient of a drug between the aqueous domain and water may be less sensitive to the change in PC_{oct} . Recently, Raykar et al. examined the uptake of a series of hydrocortisone esters varying in lipophilicity from water into untreated and delipidized human stratum corneum.⁶⁰⁾ They reported that the uptake of relatively hydrophilic solutes ($\log PC_{oct} < 3$) is governed by the protein domain, which is polar and less sensitive to changes in solute structure, while more lipophilic solutes ($\log PC_{oct} > 3$) reside preferentially in the lipid domain

which appears to have polarity similar to octanol.

The activity of the enhancers in the aqueous system was more remarkable than in the ethanolic system. The more different the polarities of the two phases is, the larger variation in the partition coefficient is. The polarity of ethanol is closer to that of the skin with or without the enhancer pretreatment than that of water is, which seems to cause the less activity of the enhancers in the ethanolic system.

The partition parameter in the ethanolic system with nonpretreated or ethanol pretreated skin diminished according to the increase in PC_{oct} . Also the amounts of drug penetrating and accumulating in the skin tended to decrease with the increase in PC_{oct} . These relationships are contrary to those observed for the aqueous system and suggest that the skin in the present experimental system had the lipophilicity less than ethanol. Waranis and Sloan reported the relationships between solubility parameter of prodrugs of 6-MP and permeability coefficient (K_p) through excised skin with three vehicles.⁶¹⁾ The increase in the solubility parameter, namely, the decrease in its lipophilicity, of prodrug increased K_p for isopropyl myristate vehicle, decreased K_p for water vehicle, and little affected K_p for propylene glycol vehicle. This can be explained if the skin had as a whole a solubility parameter, namely polarity, close to that of propylene glycol, which is more hydrophilic than ethanol.

The diffusivity of a drug in a simple system is inversely proportional to the cube root of its molar volume.⁶²⁾ There may be some interaction with a penetrant and the components of the skin such as hydrogen bonding, which makes the relationship between the molecular size and diffusivity in the skin rather complex. In the nonpretreated and ethanol pretreated control experiments, the D' value of 5-FU, the smallest molecule in this study, was the largest in

the aqueous and ethanolic systems, and those of IM and TMA, the largest molecules, were small, suggesting the dominant effect of the molecular size on the diffusivity. Ethanol pretreatment increased the D' value in almost all cases. This may be caused by the direct effect of ethanol on the skin integrity^{34,35}) and by the hydration of the skin because of the continuous wash of dermal side with saline.³¹⁻³³)

Table X summarizes the effect of the pretreatment with ethanol or the enhancers on the percutaneous drug penetration. The effect of the ethanol pretreatment was evaluated by comparing with the nonpretreated control and that of the enhancers was evaluated by comparing with the ethanol pretreated control. The cases of BP were omitted because of low reliability in the analysis based on the one-layer skin model. The penetration of drug through the ethanol pretreated skin from water was increased because of the increase in the drug diffusivity. It is clear that the pretreatment with the enhancers affected the drug affinity to the skin and altered the penetration and accumulation in the skin of the penetrant. The change in the diffusivity has less importance for the activity of the enhancers. One of mechanisms of these enhancers to enhance the penetration of above drugs is to increase the affinity of drug to the skin by changing the nature of skin as a solvent.

The penetration and skin accumulation of AC, 5-FU, and 6-MP were raised with the amount of 7GU, which was theoretically understood based on the one-layer skin model. However, Fig. 27E suggests that the penetration of BP is hardly understood by this model. It was reported for the percutaneous penetration of n-alkanols that the stratum corneum provides the rate-limiting step in the penetration process, but the permeation resistance of this layer is reduced with an increase in the alkyl chain length of alkanol.^{34,63}) The relative importance of the lower layer (viable epidermis and dermis) for

Table X. Summary of the Effect of Ethanol or Percutaneous Penetration Enhancers on the Percutaneous Penetration of Drugs^a

	Penetration through Skin	Accumulation in Skin	Diffusivity in Skin	Affinity to Skin
<u>Aqueous System</u>				
Ethanol ^b	↑	±	↑	±
Enhancer ^c	↑↑	↑	±	↑↑
<u>Ethanollic System</u>				
Ethanol ^b	↓	↓	↑	↓
Enhancer ^c	↑ or ±	↑ or ±	↑	↑ or ±

^a Effect of ethanol or the enhancer is denoted as: (↑↑) greatly increased, (↑) increased, (±) not affected, (↓) decreased.

^b Effect of the pretreatment with ethanol was evaluated by comparing with each of the nonpretreated controls.

^c Effect of the pretreatment with the enhancer was evaluated by comparing with each of the ethanol pretreated controls.

barrier properties of the whole skin rises rapidly with increasing alkyl chain length, namely, lipophilicity. Therefore, it is required to consider the diffusional resistance of the viable epidermis and dermis in considering the penetration of BP, the most hydrophobic drug in this study. The increase in the amount of BP in the skin by the enhancer is considered to be resulted from the increased affinity of BP to the stratum corneum as the other drugs in the aqueous system. The partitioning experiments showed that the enhancers increase the affinity of BP to the organic layer. The results of simulations shown in Figs. 30 and 31 suggest that the affinity of BP to the stratum corneum is too high to move into the hydrophilic layer and the penetration is decreased in spite of the increased skin accumulation.

The relationship described in Figs. 30 and 31 may widely hold in the percutaneous penetration phenomena. In some cases, a parabolic relationship is observed between the percutaneous penetration rate of homologues and their alkyl chain length.⁶⁴⁾ Such a relationship can be directly predicted by the two-layer skin model.

3.5. Summary

The percutaneous penetration enhancing effect of Azone, 7GU, and 7FU was investigated using seven penetrants with a wide range of partition coefficient. The penetration of the drugs from water vehicle (aqueous system) and ethanol vehicle (ethanolic system) through excised guinea pig skin was increased by the pretreatment with the enhancers. Enhancement was remarkable for the drugs such as 5-FU and 6-MP with n-octanol/water partition coefficient of about unity. The penetration profiles were analyzed based on the one-layer skin model, and two parameters corresponding to the drug

diffusivity and partitioning into the skin were obtained. In the aqueous system, the partitioning of drugs into the skin was increased by the enhancers, which led to the increase in the drug penetration and accumulation in the skin, while their diffusivity was little affected. In the ethanolic system, the enhancement was far less remarkable than that observed in the aqueous system because of less increase in the affinity of drug to the skin by the enhancers. From these parameters, the drug amounts in the vehicle and the skin were well estimated for the drugs with partition coefficient of lower than unity.

Further, the effect of applied dose of 7GU was investigated using four penetrants, AC, 5-FU, 6-MP, and BP. The excised guinea pig skin was preloaded with various amounts ($3.2\text{--}51.0\text{ }\mu\text{mol}/3.14\text{ cm}^2$) of 7GU, to which the aqueous solution of a drug was applied. The penetration and accumulation in the skin of AC, 5-FU, and 6-MP increased with an increase in the preloading amount of 7GU. It was suggested that the increased affinity of these drugs to the skin by 7GU was the dominant factor for the enhanced drug penetration and accumulation in the skin. For BP, larger amount of it accumulated in the skin preloaded with larger amount of 7GU, while the penetrating amount was reduced. This was the case which is not predicted by the one-layer skin model. The simulations based on a two-layer skin model, which considers the skin to be composed of stratum corneum and the lower layer, suggested that markedly large affinity of BP to the stratum corneum led to the increase in the skin accumulation and the decrease in the penetration.

The two-layer skin model was demonstrated to be adequate for the analysis of percutaneous penetration of a wide range of drugs, while the one-layer skin model holds for relatively hydrophilic penetrants.

Chapter 4. Relationship between Physicochemical Properties of Vehicle and Efficacy of Enhancer

In Chapters 2 and 3, the relationship between the structure and activity of polyprenylazacycloalkanone derivatives as percutaneous penetration enhancer and that between the physicochemical properties of penetrant and enhancing activity were elucidated. In these basic experiments, the activity of enhancers was evaluated by the pretreatment method which avoids the effect of vehicle and makes it possible to examine the direct effect of enhancers on skin. In order to achieve practical application of these enhancers, further study will be required to elucidate the effect of vehicle on the activity, because the enhancers are formulated with a vehicle in the clinical uses.

The choice of vehicle is one of the important factors which determine the activity of a percutaneous penetration enhancer.⁶⁵⁾ The enhancement of metronidazole permeation through human skin by 1 % of 1-dodecylazacycloheptan-2-one (Azone) was reported to be increased by the addition of 18 % of propylene glycol to the vehicle (ethanol) but was decreased by the addition of 18 % of polyethylene glycol 400.⁶⁶⁾ The studies in Chapter 1 showed the enhanced penetration of mitomycin C through rat skin by Azone in ethanol,^{9,10)} whereas no enhancement was observed when Azone was dissolved in isopropyl myristate vehicle. The physicochemical properties of vehicle may affect the transfer of a drug,^{67,68)} and also an enhancer from vehicle to skin, resulting in a different enhancing activity. Also some vehicles directly affect the barrier properties of skin and consequently change the penetration rate of a drug.⁶⁹⁻⁷¹⁾ In this chapter, Azone, 1-geranyl- (7GU) and 1-farnesylazacycloheptan-2-one (7FU), which had been shown to have an excellent enhancing activity, and 1-geranylazacyclopentan-2-one (5GU) were formulated

using different vehicles and examined their effect on the penetration of acyclovir (AC).

AC has marked antiviral activity in herpes virus infections, associated with low host toxicity.⁷²⁾ Intravenous, oral, and topical formulations of AC have been introduced, however, the limitation in the topical therapy of recurrent cutaneous herpes simplex virus infections has been documented.⁷³⁾ Therefore, the application of enhancers was tried for this drug.

In the following experiment, AC was applied in the form of suspension to avoid the effect of enhancer and vehicle on the thermodynamic activity of AC. The disappearance of the enhancer from vehicle was also determined and discussed in relation to the extent of the enhancing activity.

4.1. Experimental

4.1.1. Materials

AC was a gift from Sumitomo Pharmaceutical Ltd., Japan. Four solvents, propylene glycol (PG), ethanol (ET), isopropanol (IPA), and isopropyl myristate (IPM) were purchased from Nacalai Tesque, Inc., Japan and employed as vehicles. Azone was kindly supplied from Nelson Research Center, USA, and 7GU, 7FU, and 5GU were synthesized by Kuraray Co. Japan.

4.1.2. In Vitro Percutaneous Penetration Experiment

Transdermal delivery rates of AC were determined by in vitro diffusion experiments. The full-thickness dorsal skin of female hairless mice (9-10 weeks) was excised in one piece and adherent fat and other visceral debris were removed from the undersurface. Also the full-thickness of abdominal skin of male Wistar rats (ca. 300 g) was obtained after the removal of the

hair, and adipose tissue was detached. The freshly excised skin was mounted on a diffusion cell with an available diffusion area of 8.04 cm^2 (Fig. 2). The receptor compartment of the apparatus was filled with 48 ml of saline containing streptomycin sulfate (50 mg/l, Sigma Chemical Co.) and penicillin G potassium salt (30 mg/l, Toyo Jozo, Japan), and stirred with a magnetic stirrer. Test formulations were prepared by suspending AC in PG, ET, IPA, and IPM to a total concentration of 20 mM. The enhancers were added to these suspensions at a concentration of 0.1 M. The AC suspension (2 ml) was applied to the donor compartment, which was sealed with silicone stopper. The diffusion cell was thermostated at 32°C in a water bath. At appropriate intervals, 1 ml of the receptor medium was withdrawn and this volume was replaced with fresh medium. At the end of experiment, the drug and enhancer remaining in the donor phase were recovered with methanol. Twenty-five milliliter of methanol was sufficient to dissolve AC, enhancer, and vehicle.

AC was determined using the HPLC system (TRI ROTAR, Japan Spectroscopic Co., Ltd.) in a reverse-phase mode equipped with a UV detector (UVIDEC-100-III, Japan Spectroscopic Co. Ltd.) operating at 252 nm. The mobile phase was a mixture of methanol and distilled water (10 : 90) and flowed at 0.8 ml/min. The sample from receptor compartment was analyzed after filtration with Milliporefilter (pore size, 0.45 μm). The sample obtained from the donor compartment (methanol solution) was analyzed after evaporation of methanol and resolubilization in water.

The enhancers recovered from donor phase were determined by HPLC system with a UV detector operating at 210 nm. The mobile phase was a mixture of methanol and water (90 : 10) and flowed at 1.0 ml/min.

4.2. Results

4.2.1. Lipophilicity of Enhancers and Vehicles

Table XI lists the lipophilic indices (see Chapter 1) and solubility parameters calculated by the method of Fedors of enhancers and vehicles.¹⁶⁾ The solubility parameters of the enhancers (9.07–9.30) are closer to that of IPM (8.54) than to the other vehicles (11.6–14.8). The solubility parameters inversely follow the order of the determined lipophilic indices in enhancers. The solubility parameters of vehicles show an order of the polarity of vehicles predicted from their chemical structures.

4.2.2. Effect of Vehicle on Activity of Enhancer to Penetration of AC

The penetration profiles of AC through hairless mouse skin from IPA and IPM are illustrated in Figs. 32A and B, respectively. The penetration of AC from IPA was remarkably increased by 7GU, whereas that from IPM was not affected, suggesting the importance of vehicle for the activity of the enhancer.

The enhancing effect of the enhancers formulated in the four vehicles was examined using excised rat skin. Figures 33A–D show the penetration profiles of AC through rat skin from PG, ET, IPA, and IPM vehicles with or without the enhancers. Table XII summarizes the recovery percentages of AC and enhancers at the end of 8-h diffusion experiment.

Theoretically, all vehicles containing finely ground suspensions of the drug will produce the same rate of penetration because the thermodynamic activity of the drug in them is equal to that of the solid drug⁵⁷⁾. When results of control experiments were compared, however, the AC penetration from the vehicle with higher lipophilicity was faster (IPM > IPA > ET > PG). Also

Table XI. Physicochemical Properties of Percutaneous Penetration Enhancers and Vehicles

Compound	Solubility Parameter ^a (cal/cm ³) ^{1/2}	Lipophilic Index
Azone	9.07	6.14
7FU	9.10	5.86
7GU	9.21	3.86
5GU	9.30	3.30
Propylene Glycol (PG)	14.8	ND ^b
Ethanol (ET)	12.6	ND ^b
Isopropanol (IPA)	11.6	ND ^b
Isopropyl Myristate (IPM)	8.54	7.75

^a Estimated value by the method of Fedors.¹⁶⁾

^b Not determined.

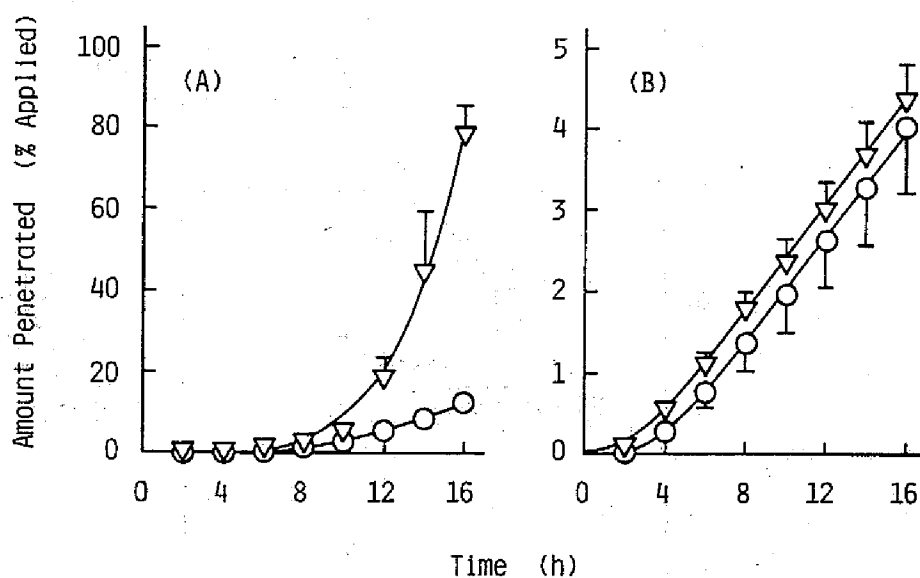


Figure 32. Penetration of AC through the excised hairless mouse skin. AC was applied as suspensions in IPA (A) and IPM (B) with (▽) and without (○) 7GU.

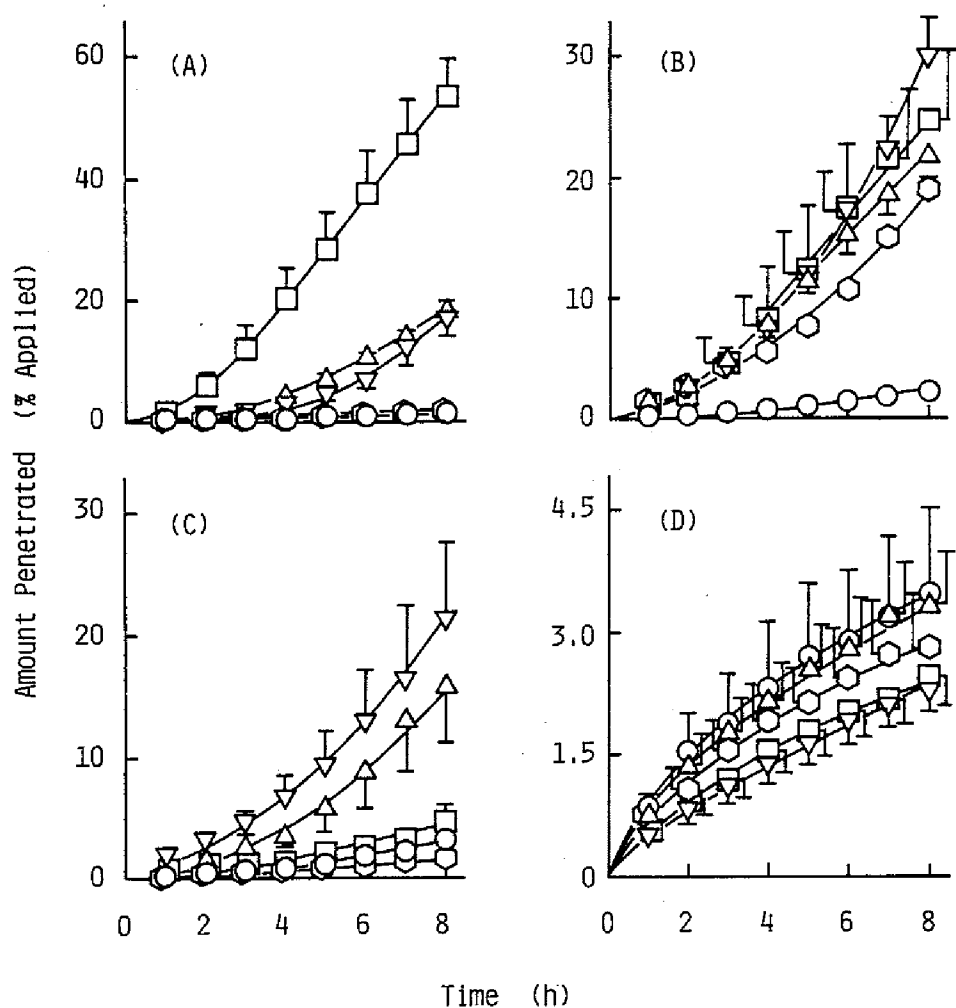


Figure 33. Penetration of AC from PG (A), ethanol (B), IPA (C), and IPM (D) through excised rat skin. AC was suspended in the vehicle without enhancer (○) or with Azone (□), 7FU (△), 7GU (▽), and 5GU (◇).

Table XII. Recovery Percentages of Enhancers and AC after 8-Hour Diffusion Experiments with Rat Skin^a

Vehicle	Enhancer	N	Recovery from Donor (%)		Recovery of AC from Receptor (%)
			Enhancer	AC	
PG	Control	3	—	103.86 ± 4.98	1.30 ± 0.43
	Azone	3	89.03 ± 4.32	44.64 ± 9.79	53.78 ± 5.79
	7FU	3	86.65 ± 1.35	79.33 ± 2.21	18.38 ± 1.46
	7GU	3	82.75 ± 4.46	79.01 ± 16.66	16.66 ± 2.62
	5GU	3	88.43 ± 1.60	105.82 ± 7.20	2.07 ± 0.66
ET	Control	3	—	104.76 ± 7.11	2.17 ± 0.37
	Azone	3	91.69 ± 3.14	58.16 ± 7.08	24.39 ± 5.91
	7FU	3	84.71 ± 6.10	71.93 ± 6.46	21.81 ± 2.01
	7GU	3	87.39 ± 0.86	60.77 ± 6.39	29.53 ± 3.32
	5GU	3	84.06 ± 2.14	68.73 ± 2.33	18.77 ± 0.34
IPA	Control	3	—	91.13 ± 2.43	3.13 ± 0.96
	Azone	4	87.72 ± 1.65	101.60 ± 4.40	4.83 ± 1.24
	7FU	3	86.47 ± 3.28	72.04 ± 6.86	15.90 ± 4.83
	7GU	3	65.91 ± 25.06	73.97 ± 21.93	21.18 ± 6.41
	5GU	3	89.05 ± 4.26	94.63 ± 7.46	1.63 ± 0.32
IPM	Control	3	—	87.85 ± 14.66	3.44 ± 1.08
	Azone	4	91.61 ± 2.17	93.17 ± 3.93	2.43 ± 0.33
	7FU	4	89.90 ± 1.46	96.29 ± 4.60	3.34 ± 0.64
	7GU	3	92.66 ± 1.51	96.29 ± 4.60	2.26 ± 0.22
	5GU	3	90.02 ± 2.19	91.86 ± 6.29	2.80 ± 0.66

^a Data are expressed by means ± standard errors of the mean.

the amount of AC remaining in the hydrophobic vehicle was smaller than that in the hydrophilic vehicle. Thus, vehicle itself affected the permeability of the skin to some extent. However the variation of AC penetration observed in different vehicles was much smaller than that in different enhancers.

The variation of control values suggests the direct effect of the vehicles on the skin permeability. To normalize the contribution of the direct effect of vehicles, the ratio of the amount of AC penetrating for 8 h from a vehicle with an enhancer to that from the same vehicle without enhancers (increasing ratio in AC penetration) was calculated and is plotted against the solubility parameter of the vehicle and lipophilic index of the enhancer in Fig. 34. The brief examination of this figure reveals that the activity of enhancer tends to be encouraged in the vehicle with high hydrophilicity (with larger solubility parameter value). This phenomenon was more remarkable for the enhancer with higher lipophilicity. The effect of 5GU was large in ET. 7GU showed the large effect in PG and ET. The effect of 7FU and Azone was increased according to the increase in hydrophilicity of the vehicle and the maximum enhancement in AC penetration was observed in the combination of PG and Azone, the most hydrophilic vehicle and the most lipophilic enhancer. IPM was the unfavorable vehicle for all enhancers examined. In IPA, the enhancer with the lipophilic index of 4 (7GU) seems to show the greatest activity, while all enhancers showed almost the same enhancing activity in ET. PG seems to raise the activity of enhancer with higher lipophilicity.

During the 8-h diffusion experiments, about $2-4 \mu\text{mol}/\text{cm}^2$ of enhancer was disappeared from the vehicle. The disappearance of the enhancer from the vehicle basically correlated with the enhancing activity (Table XII). The activity of 5GU was demonstrated when formulated in ET, from which 5GU

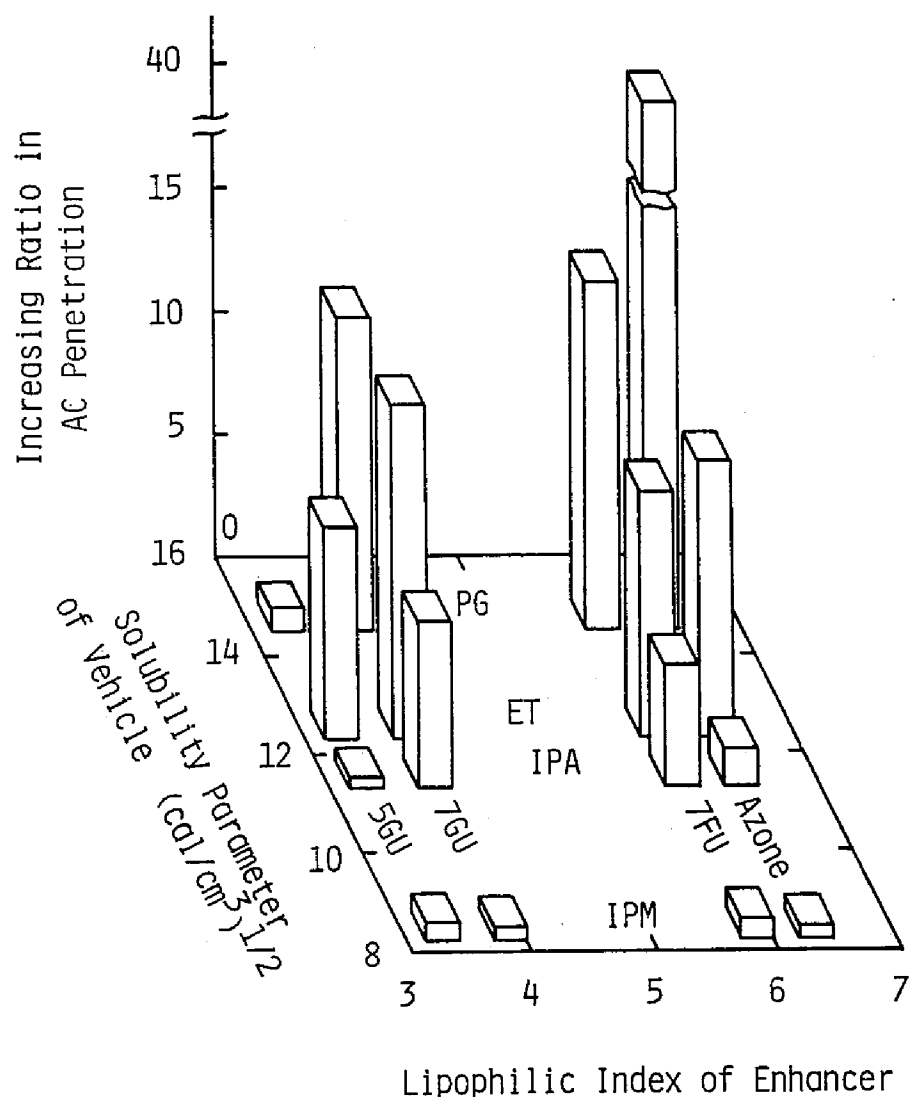


Figure 34. Effect of Azone, 7FU, 7GU, and 5GU on the percutaneous penetration of AC from various vehicles with different physicochemical properties.

disappeared more rapidly than the other vehicles. IPM, which caused little activity of enhancers, showed larger remaining amount of the enhancers in it. However the drastic enhancement of AC penetration did not always follow the large disappearance of the enhancer (for instance, the combination of Azone and PG), suggesting the existence of factors other than penetration of enhancer itself that determines the activity of the enhancers.

4.3. Discussion

The physicochemical properties of vehicle, drug, and skin are major three determinants for percutaneous penetration of drug.⁷⁾ Some changes in one of them alter the dynamic interactions developing between them and affect the percutaneous drug penetration.

The effect of enhancer was remarkably affected by the vehicle. The penetration of AC was reported to be increased by increasing the amount of 7GU (Chapter 3).⁵²⁾ It is possible that vehicles alter the movement of enhancer itself to skin. The enhancers were not saturated in the vehicle and their thermodynamic activity is varied in different vehicles. Sloan et al. investigated the penetration of theophylline,⁶⁹⁾ salicylic acid,⁷⁰⁾ and 6-mercaptopurine⁷¹⁾ in various vehicles and demonstrated that the flux and permeability coefficient were minimized when the solubility parameter of vehicle was close to that of penetrant. The penetration enhancers in this study have the solubility parameters close to that of IPM, which leads to relatively small penetration of enhancers and reduction in their enhancing activities.

IPM caused somewhat unusual diffusion profiles of AC. One of the reasons of this may be that the rate limiting step was not in the passage

through the skin but in the applied phase. It is possible that the dissolving rate of suspended AC determined the penetration rate. However, the suppressed activity of the enhancers in IPM was suggested by the facts that no effect of them was observed in even the early stage of the diffusion and no effect of 7GU was demonstrated in the experiment with hairless mouse skin in which normal penetration profiles were obtained. The great enhancing effect observed in the combinations of relatively hydrophilic vehicle and hydrophobic enhancer is not fully explained by the extent of enhancer's penetration. Keeping the idea that the thermodynamic activity of AC in the vehicle was not varied, the direct effect of vehicle on the skin is speculated. It has been reported that PG encouraged the percutaneous penetration enhancing activity of oleic acid⁵⁵⁾ lauric acid, and lauryl alcohol.³⁷⁾ Barry proposed the synergistic action of PG and Azone,⁷⁴⁾ which may be the case for AC penetration. The decrease in the amount of PG from the donor cell was obvious at the end of diffusion experiment when Azone was added to it. In spite of the notable improvement of AC penetration, the disappearance of enhancers from PG was not so large as expected from the difference of their solubility parameters. The penetration of hydrophilic vehicle into the stratum corneum seems to make the polarity of skin larger, which results in a decrease in affinity of the enhancers to the skin.

4.4. Summary

The effect of vehicle and percutaneous penetration enhancer on the penetration of AC through excised hairless mouse and rat skin was investigated. Four kinds of solvents, PG, ET, IPA, and IPM, were employed as vehicles and four enhancers, 5GU, 7GU, 7FU, and Azone were formulated in them.

AC was suspended in vehicles to avoid the effect on the thermodynamic activity of AC in the vehicle. All of the combinations of a vehicle and a penetration enhancer were examined using rat skin. No enhancing effect of the enhancers was observed for IPM vehicle. On the other hand, the penetration of AC from the other vehicles was raised with the enhancers. In hairless mouse skin, the penetration of AC from IPA was also enhanced by 7GU, whereas that from IPM was not changed. From the examination of solubility parameters of vehicles and enhancers, it was suggested that the hydrophobicity of IPM is close to those of enhancers and this might lead to the decrease in the release of the enhancers from IPM. The combination of hydrophilic vehicle and the hydrophobic enhancer resulted in the large enhancing effect. The disappearance of the enhancers from the vehicle seemed to correlate with the enhancing activity. However, other factors also seemed to affect the great enhancement of penetration of AC.

Summary and Conclusions

Polyprenylazacycloalkanone derivatives were developed as novel percutaneous penetration enhancers and their activities were examined in in vitro diffusion experiments with excised animal skin. The effect of the enhancers was discussed in relation to three dominant determinants in percutaneous penetration of drugs, namely, physicochemical properties of drug, those of vehicle, and nature of skin.

The drug penetration through the skin is, in general, considered as the passive diffusion process, and mathematical analysis based on the diffusion theory seems to be effective to correlate drug movement with its physicochemical properties. Two diffusion models were constructed in this study which reflect the experimental conditions with the flow-through type diffusion cell. The Laplace transformed equations describing the percutaneous penetration process were derived and the obtained percutaneous penetration profiles were analyzed by the aid of MULTI(FILT) which is a nonlinear least squares computer program based on fast inverse Laplace transform algorithm.

Through the present studies, it is concluded on following six points as below.

1) Evaluation of Activity of Polyprenylazacycloalkanone Derivatives as Percutaneous Penetration Enhancers

- 1) Polyprenylazacycloalkanone derivatives enhance significantly percutaneous penetration of drugs.
- 2) These compounds directly affect skin permeability.
- 3) These compounds have less skin irritancy than Azone.

2) The Relationship between the Structure and Activity of the Penetration Enhancer

- 1) Tail chain length has important effect on the enhancing activity. C10 or C15 seems to be favorable.
- 2) The number of carbonyl groups in a ring moiety of enhancer has important effect on the activity. Enhancers with one carbonyl group are better than those with two.
- 3) Trans double bonds in tail chain cause the less skin irritancy.

3) The Relationship between the Physicochemical Properties of Penetrant and Efficacy of Enhancer

- 1) Enhancement is remarkable for the drugs with n-octanol/water partition coefficient of about unity.
- 2) The dominant action of the developed enhancers is to increase the drug partitioning to skin and drug concentration gradient in skin barrier.

4) The Relationship between the Applied Dose of Enhancer and Its Efficacy

- 1) Drug penetrability rises with the dose of enhancer because of an increase in affinity to the skin.
- 2) Large increase in lipophilicity of penetrant results in an increased accumulation in the stratum corneum and a decreased penetration because the drug movement from the stratum corneum to the lower layer becomes a rate-limiting step.

5) The Relationship between the Physicochemical Properties of Vehicle and Efficacy of Enhancer

- 1) The combination of hydrophilic vehicles such as propylene glycol and ethanol and hydrophobic enhancers results in the large enhancing effect.
- 2) The disappearance of the enhancer from the vehicle seems to correlate with the enhancing activity.

6) Development of Diffusion Model for Analyzing and Predicting Percutaneous Penetration Process

- 1) One-layer skin model is valid for the analysis of the penetration of relatively hydrophilic drug.
- 2) Two-layer skin model is useful to analyze the percutaneous penetration process of a wide range of drug in physicochemical terms such as diffusion constant and partition coefficient.
- 3) The concept of mean transit time is helpful to grasp the percutaneous penetration process.

The experimental system and analytical method developed in this work can be widely applied in studies of percutaneous penetration and will give useful information in physicochemical terms. The results obtained in this study should be a valuable guide for development of novel percutaneous penetration enhancers and formulation of them.

Appendix

1) Laplace Transformed Equations Based on One-Layer Skin Model

The corresponding Laplace transforms of Eqs. 3-7 are:

$$s\bar{C} = D \frac{\partial^2 \bar{C}}{\partial x^2} \quad (A1)$$

$$\bar{C} = 0 \quad (\text{at } t = 0, 0 < x \leq L) \quad (A2)$$

$$\bar{C}_1 = 0 \text{ or } \bar{C}_1 V_1 = X_0 \quad (t = 0, -V_1/A \leq x \leq 0) \quad (A3)$$

$$\frac{V_1}{K} s\bar{C} = X_0 + DA \left(\frac{\partial \bar{C}}{\partial x} \right)_{x=0} \quad (\text{at } x = 0) \quad (A4)$$

$$\bar{C} = 0 \quad (\text{at } x = L) \quad (A5)$$

Equation A1 has the general solution:

$$\bar{C} = A' \cosh(qx) + B' \sinh(qx) \quad (A6)$$

where:

$$q = (s/D)^{1/2} = d/L \quad (A7)$$

From Eqs. A2-A7, A' and B' are obtained as:

$$A' = KX_0 \sinh(qL)/U' \quad (A8)$$

$$B' = -KX_0 \cosh(qL)/U' \quad (A9)$$

where:

$$U' = s \cdot V_1 \cdot \sinh(qL) + KDAq \cdot \cosh(qL) \quad (A10)$$

The total amounts of drug appearing in the receptor (Q), accumulating in the skin (M), and remaining in the donor (X) are:

$$Q = -DA \int_0^t \left(\frac{\partial C}{\partial x} \right)_{x=L} dt \quad (A11)$$

$$X = X_0 + DA \int_0^t \left(\frac{\partial C}{\partial x} \right)_{x=0} dt \quad (A12)$$

$$M = A \int_0^L C dx \quad (A13)$$

The Laplace transform for the total drug amounts in the receptor (\bar{Q} , Eq. 8), in the donor (\bar{X} , Eq. 9), and in the skin (\bar{M} , Eq. 10) are given as:

$$\bar{Q} = - \frac{DA}{s} \left(\frac{\partial \bar{C}}{\partial x} \right)_{x=L} = - \frac{DA}{s} q [A' \sinh(qL) + B' \cosh(qL)] \quad (A14)$$

$$\bar{X} = \frac{X_0}{s} + \frac{DA}{s} \left(\frac{\partial \bar{C}}{\partial x} \right)_{x=0} = \frac{X_0}{s} + \frac{DA}{s} q B' \quad (A15)$$

$$\bar{M} = A \int_0^L \bar{C} dx = \frac{A}{q} \{A' \sinh(qL) + B' [\cosh(qL) - 1]\} \quad (A16)$$

When the Laplace transformed equation for the cumulative amount of a drug penetrating a barrier (\bar{Q}) is given as a function of the Laplace variable (s) as Eq. A17:

$$\bar{Q} = \frac{h(s)}{sg(s)} \quad (A17)$$

the area under the curve (AUC) and the area under the first moment curve (AUMC) are obtained as:

$$AUC = \lim_{s \rightarrow 0} (s\bar{Q}) = \lim_{s \rightarrow 0} \frac{h(s)}{g(s)} \quad (A18)$$

$$AUMC = -\lim_{s \rightarrow 0} \left\{ \frac{d}{ds} (s\bar{Q}) \right\} = -\lim_{s \rightarrow 0} \left\{ \frac{d}{ds} h(s)/g(s) - h(s) \frac{d}{ds} k(s)/g(s)^2 \right\} \quad (A19)$$

The MTT is calculated by:

$$MTT = \frac{AUMC}{AUC} = \lim_{s \rightarrow 0} \left\{ \frac{d}{ds} g(s)/g(s) - \frac{d}{ds} h(s)/h(s) \right\} \quad (A20)$$

2) Laplace Transformed Equations Based on Two-Layer Skin Model

The corresponding Laplace transforms of Eqs. 16-24 are, respectively:

$$s\bar{C}_2 = D_2 \frac{\partial^2 \bar{C}_2}{\partial x^2} \quad (A21)$$

$$s\bar{C}_3 = D_3 \frac{\partial^2 \bar{C}_3}{\partial x^2} \quad (A22)$$

$$K_{12}\bar{C}_1 = \bar{C}_2 \quad (x = -L_2) \quad (A23)$$

$$sV_1\bar{C}_1 - X_0 = D_2 A \frac{\partial \bar{C}_2}{\partial x} \quad (x = -L_2) \quad (A24)$$

$$K_{23}\bar{C}_2 = \bar{C}_3 \quad (x = 0) \quad (A25)$$

$$D_2 \frac{\partial \bar{C}_2}{\partial x} = D_3 \frac{\partial \bar{C}_3}{\partial x} \quad (x = 0) \quad (A26)$$

$$\bar{C}_3 = 0 \quad (x = L_3) \quad (A27)$$

Eqs. A21 and A22 have the general solutions, respectively:

$$\bar{C}_2 = X' \cosh(q_2 x) + Y' \sinh(q_2 x) \quad (A28)$$

$$\bar{C}_3 = Z' \cosh(q_3 x) + W' \sinh(q_3 x) \quad (A29)$$

where:

$$q_2 = (s/D_2)^{1/2} \quad (A30)$$

$$q_3 = (s/D_3)^{1/2} \quad (A31)$$

From Eqs. A23-A31, X' , Y' , Z' and W' are determined, respectively, as:

$$X' = Z'/K_{23} \quad (A32)$$

$$Y' = (D_3/D_2)^{1/2} W' \quad (A33)$$

$$Z' = -\tanh(q_3 L_3) W' \quad (A34)$$

$$W' = \frac{-K_{12} K_{23} X_0}{\tanh(q_3 L_3) Q' + K_3 (D_3/D_2)^{1/2} R'} \quad (A35)$$

where:

$$Q' = sV_1 \cosh(q_2 L_2) + K_{12} D_2 A q_2 \sinh(q_2 L_2) \quad (A36)$$

$$R' = sV_1 \sinh(q_2 L_2) + K_{12} D_2 A q_2 \cosh(q_2 L_2) \quad (A37)$$

\bar{Q} , \bar{X} , \bar{M}_2 , and \bar{M}_3 are calculated, respectively, by:

$$\bar{Q} = -\frac{D_3 A}{s} \left(\frac{\partial \bar{C}_3}{\partial x} \right)_{x=L_3} = -\frac{D_3 A}{s} q_3 [Z' \sinh(q_3 L_3) + W' \cosh(q_3 L_3)] \quad (A38)$$

$$\bar{X} = \frac{X_0}{s} + \frac{D_2 A}{s} q_2 \left(\frac{\partial \bar{C}_2}{\partial x} \right)_{x=-L_2} = \frac{X_0}{s} + \frac{D_2 A}{s} q_2 [-X' \sinh(q_2 L_2) + Y' \cosh(q_2 L_2)] \quad (A39)$$

$$\bar{M}_2 = A \int_{-L_2}^0 \bar{C}_2 dx = \frac{A}{q_2} \{X' \sinh(q_2 L_2) - Y' [\cosh(q_2 L_2) - 1]\} \quad (A40)$$

$$\bar{M}_3 = A \int_0^{L_3} \bar{C}_3 dx = \frac{A}{q_3} \{Z' \sinh(q_3 L_3) + W' [\cosh(q_3 L_3) - 1]\} \quad (A41)$$

MTT (Eq. 32) is calculated by the same manner as in the one-layer skin model, by substituting $g(s)$ in Eqs. A17-A19 with $k(s)$.

The Laplace transformed equations for the total drug amount penetrating the boundary at 0 (\bar{Q}_{12}) and $-L_2$ (\bar{Q}_{23}) are derived as the same manner as \bar{Q} :

$$\bar{Q}_{12} = K_{12}K_{23}V_2V_3X_0\cosh(d_3)/s/k(s) \quad (A42)$$

$$\bar{Q}_{23} = K_{12}V_2X_0[V_2d_3/d_2\sinh(d_2)\sinh(d_3) + K_{23}V_3\cosh(d_2)\cosh(d_3)]/s/k(s) \quad (A43)$$

MTT for an applied penetrant to traverse the boundary at $x=-L_2$, between the donor and the stratum corneum, (MTT_{12}) and that at $x=0$, between the stratum corneum and the lower layer, (MTT_{23}) are given as follows:

$$MTT_{12} = \frac{V_1/K_{12}}{D_2'V_2} + \frac{V_1/K_{12}K_{23}}{D_3'V_3} \quad (A44)$$

$$MTT_{23} = MTT_{12} + \frac{1}{2D_2'} + \frac{V_2/K_{23}}{D_3'V_3} \quad (A45)$$

MTT_{12} is equal to t_1 . The last two terms in Eq. A45 may be divided into the two terms, one of which is determined only by the nature of the stratum corneum ($t_2 = 1/2D_2'$) and the other is affected by the nature of the lower layer (t_3). The comparison of Eqs. 31 and A45 reveals that the $1/2D_3'$ is the MTT for the penetrant to diffuse in the lower layer (t_4).

3) Nomenclature

A	Effective area for diffusion
C	Drug concentration
d	$L/(s/D)^{1/2}$
D	Diffusion constant of drug
D'	Diffusion parameter (D/L^2)
K	Partition coefficient of drug
K'	Partition parameter ($K \cdot L$)
L	Thickness of membrane
M	Drug amount in the skin

MTT	Mean transit time
q	$(s/D)^{1/2}$
Q	Total amount of drug penetrating the skin
s	Laplace variable with respect to time
V	Volume
x	Distance
X	Drug amount in the donor solution

(Subscripts)

0	Initial condition
1	Donor solution
2	Stratum corneum or skin
3	The lower layer (viable epidermis + dermis)
12	Boundary between the donor solution and the stratum corneum
23	Boundary between the stratum corneum and the lower layer

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